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UNIVERSITÉ DE MONTRÉAL

MECHANICAL PRETREATMENT OF LIGNOCELLULOSIC BIOMASS TO INCREASE THE YIELD OF ENZYMATIC HYDROLYSIS

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Ce mémoire intitulé:

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présenté par : JU Chen

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DEDICATION

This thesis is gratefully dedicated to

my loving husband and my beloved father and mother.

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RÉSUMÉ

Le sucre est une matière première importante qui est issue d'opérations de bioraffinage. Sa conversion en biocarburants ou en produits chimiques représente une alternative aux produits dérivés de ressources fossiles. Cependant, la biomasse lignocellulosique fait preuve de résistance à l'hydrolyse enzymatique, ce qui rend difficile sa conversion en sucres. Le développement d'un procédé de prétraitement efficace préalable à cette opération de saccharification représente un défi pour d'en augmenter l'efficacité.

Le raffineur à disques qui'était utilisée dans les usines de pâtes mécaniques, la baisse de la demande en la matière en a provoqué l'inactivité forcée. La technologie de raffinage à disques serait intéressante pour le prétraitement de la biomasse lignocellulosique car elle permet d'en augmenter la digestibilité enzymatique grâce à la réduction de la taille de ses particules et à l'augmentation de la surface de ses fibres. Son application dans des bioraffineries permettrait de diminuer considérablement les coûts d'investissement et les risques associés.

Un système de raffinage à disques (à échelle pilote) a été modifié dans le cadre de ce projet en vue d'obtenir de hauts rendements en sucres et de faibles consommations énergétiques lors de la saccharification de différents types de biomasse lignocellulosique par hydrolyse enzymatique.

La première étape de ce projet vise à étudier un traitement thermique avec ajout de NaOH (5% massique par-rapport à la biomasse) sur de copeaux de bois. Les résidus issus de cette étape sont assujettis à différentes étapes de raffinage à disques (leur consistance varie alors de 5 à 15%, l'écart entre les disques de 0.15 à 1.00 mm) et subissent une hydrolyse enzymatique. Les résultats alors obtenus sont les suivants :

- Le traitement thermique à 140°C avec ajout de NaOH permet l'extraction partielle de la lignine et l'augmentation significative des rendements en sucres lors de l'étape de saccharification tout en diminuant la quantité d'énergie consommée lors du prétraitement mécanique.
- Le raffinage à faible consistance permet de réduire les coûts en énergie tout en diminuant le rendement en sucres de l'hydrolyse; un faible écart entre les disques augmente le rendement en sucres mais provoque une augmentation significative des coûts d'énergie.

- Le procédé de prétraitement à disques est constitué de deux étapes : un prétraitement thermochimique (TCP) suivie d'un prétraitement de raffinage à disques (DRP). Les conditions optimales déterminées consistent en l'ajout de 5% massique (par-rapport à la biomasse) de NaOH, d'un traitement à la vapeur à 140°C pendant 30 minutes, d'une consistance de raffinage de 8% et d'un écart de 0.80 mm entre les disques.
- Le procédé TCDR a un rendement en sucres 35% plus importants qu'un procédé de mise en pâte mécanique non-modifié et consomme 62% moins d'énergie.

La seconde phase de ce projet est une étude de l'application du traitement TCDR à différents types de biomasse lignocellulosique (bouleau, pin noir, tiges de maïs et luzerne). Les compositions chimiques et structures physiques sont déterminées pour chacune d'entre-elles, ainsi que les courants de sucres et les quantités d'énergies associées à leur passage dans le procédé de traitement. Les résultats alors obtenus sont les suivants:

- Le procédé de prétraitement à la vapeur permet d'extraire la lignine et les hémicelluloses pour tous les types de biomasse. Parmi tous les types de biomasses prétraitées, les tiges de maïs sont celles contenant la plus faible quantité de lignine.
- Parmi tous les types de biomasses prétraitées, pour un même écart entre les disques, les fibres issues du pin noir sont les plus longues alors que celles des tiges de maïs sont les plus courtes.
- L'opération de prétraitement a un effet plus important sur les tiges de maïs et sur la luzerne que sur le bouleau et le pin noir. Plus particulièrement, les rendements en sucres pour les tiges de maïs prétraitées sont les plus importants (97.3%), et sa consommation énergétique est de 196 kWh par tonne de biomasse.

ABSTRACT

Sugar is a core transitional biofeedstock produced in a biorefinery process that can be further converted into biofuels or chemicals thus offering a sustainable alternative to fossil fuel-based chemicals. However, one challenge of sugar production from lignocellulosic biomass is to develop an efficient pretreatment process to improve the efficiency of enzymatic hydrolysis, due to its unique lignocellulosic biomass recalcitrances.

Disk refiners usually used in mechanical pulping mills might have become idled industrial machines because of the low demand for traditional products. The disk refining technology has attracted attention for the lignocellulosic biomass pretreatment in a biochemical conversion process, since it can improve enzymatic digestibility by removing biomass recalcitrances. Also, using existing highly commercialized disk refining equipment into a biorefinery mill would significantly lower investment cost and reduce associated risks.

In this thesis, an existing disk refining system (pilot plant scale) utilized as a pretreatment process has been modified to obtain a high sugar yield by enzymatic hydrolysis with a low energy consumption for different lignocellulosic biomasses.

In the first phase, a thermal treatment with NaOH addition (5% w/w biomass) before disk refining was studied for hardwood chips. The residues were the feedstocks of the disk refining system at various disk refining gap sizes from 0.15 mm to 1.00 mm and consistency from 5% to 15%. The solid residues then were used for enzymatic hydrolysis.

- The results showed that the thermal treatment (140˚C) with NaOH addition can partially remove lignin and significantly increase sugar yield. It also reduces energy consumption of mechanical pretreatment.
- Low consistency refining can reduce energy cost and slightly decrease sugar yield. Small refining gap size increases sugar yield while results in significantly higher energy cost.
- The strategy of the thermochemical disk refining pretreatment (TCDRP) process consists of a two-step treatment: a thermochemical pretreatment (TCP) and a disk refining pretreatment (DRP). The optimum condition of TCDRP is NaOH addition of 5% (w/w dry biomass), steaming at 140˚C for 30 minutes, refining consistency at 8% and refining gap at 0.80 mm.

- Compared to a non-modified typical mechanical pulping process, the TCDRP pretreatment has higher sugar yield by 35% with an energy saving of 62%.

The second phase of this work focused on the investigation of the TCDRP on different lignocellulosic biomasses including white birch, black spruce, corn stover and alfalfa. It consists of the characterizations of chemical composition and physical structure, and analysis of energy consumption and sugar streams.

- The results revealed that there is a removal of lignin and hemicelluloses by the TCDRP for all biomasses. The corn stover pretreated by the TCDRP had the lowest lignin content versus the other biomasses pretreated by the TCDRP.
- At the same refining gap size, the TCPRP treated corn stover had the lowest fiber length while the TCDRP treated black spruce had the highest fiber length.
- According to energy consumption and sugar yield, this TCDRP had more effect on corn stover and alfalfa than on white birch and black spruce. In particular, the sugar yield of the TCDRP treated corn stover is the highest (97.3%) compared to other biomasses; its energy consumption is 196 kWh/ton.

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LIST OF SYMBOLS AND ABBREVIATIONS

CTMP	Chemithermomehchanical pulping
c	The pulp consistency (kg/L)
DRP	Disk refining pretreatment
DP	Degree of polymerization
E_{net}	The energy applied to fibers (kW)
E_d	The energy communed in the defibration stage (kW)
E_f	The energy consumed in the additional fiber modification stage (kW)
GHG	Greenhouse gas
G	Refining gap size (mm)
I	Refining intensity
M	Mass flow rate of pulp (ton/h)
N	The number of impacts
NSE	Net specific energy (kWh/ton)
n_b	The number of bars
$P_{no\text{-}load}$	The initial power or no-load power (kW)
P_{net}	The net power consumed to change the pulp properties (kW)
P_{tot}	The total power consumed (kW)
Q	The flow rate (L/min)
RMP	Refining mechanical pulping
S	Severity factor
SPORL	Sulfite pretreatment to overcome recalcitrance of lignocellulose
TCP	Thermochemical pretreatment

TCDRP	Thermochemical disk refining pretreatment
TMP	Thermomechanical pulping
t_r	Residence time (min)
T	Thermal temperature (˚C)
t	Thermal temperature holding time (min)

CHAPTER 1 INTRODUCTION

1.1 Context and motivation

Renewable energy is very critical for climate change, the independence of non-renewable energy, and energy security in future society. Currently, the dominant energy is from fossil resources such as coal, petroleum, and natural gas. Due to a continuous increase of energy demands and gradual depletion of fossil resources, the production of renewable fuels and chemicals needs increase for the long term. For example, US has a production goal of 36 billion gallons per year by 2022 [1] and European targets on 10% replacement of fossil fuels for transport at 2020 [2].

Biomass, a carbon neutral resource, has major attractions of biomass as an energy raw material because of its renewability and domestic availability in many countries [1, 3]. Biorefinery using complex processing technologies can convert biomass into biofuels, organic chemicals, polymers, and materials, with beneficial to the environment and the economy.

Sugar is a core transitional biofeedstock produced in a biochemical process, which can be further converted into biofuels or chemicals. Currently, almost all sugars are produced from sugar cane or grain. The disadvantages of these raw materials include the competition with food sources, the devastation of forests and the increase of agricultural land. According to its enriched feedstock and non-food resources, lignocellulosic biomass has attracted more and more attentions these years.

The biochemical conversion pathway of lignocellulosic biomass consists of raw material pretreatment, hydrolysis, fermentation and distillation [2]. Pretreatment is considered to be a central unit process in a biorefinery to make lignocellulosic biomass amenable to biological conversion at high yield [4, 5]. It also affects costs and yields for all other operations in the process [4]. Although many pretreatment methods were developed such as physical, chemical, combinations and others, there is still a lack of an efficient pretreatment for the commercialization of biorefineries.

In physical methods, mechanical pretreatment has a high possibility of large production capacity by using industrial devices - disk refiners. Disk refining technology have been developed over a century ago with the application for pulp and papermaking. Through disk refiner, wood chips could be broken down into fibers to get pulp yield with more than 85% [6], such as refiner mechanical pulp (RMP), thermomechanical pulp (TMP) and chemithermomechanical pulp (CTMP). The pulp

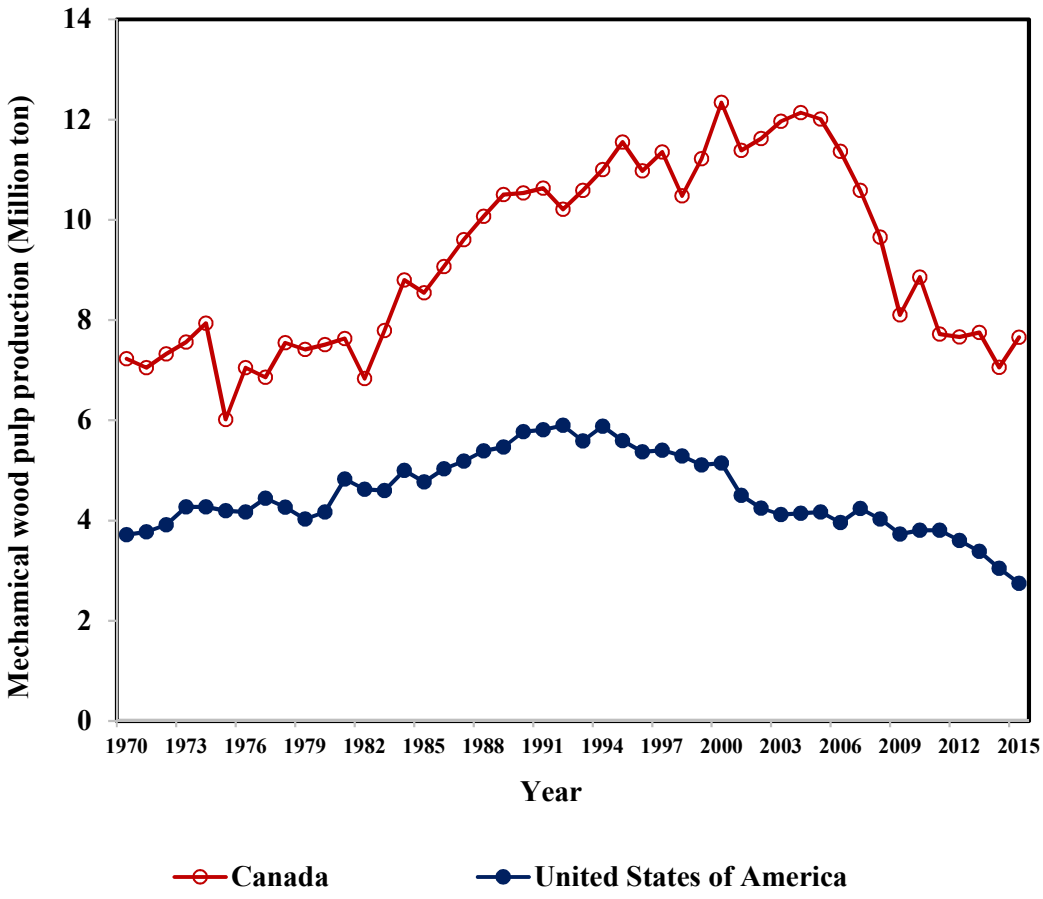

Figure 1.1: Mechanical wood pulp production [7]

is mainly used in newsprint and other writing papers [6]. However, there were about 37% of mechanical pulping production decreased since the year of 2004 (Figure 1.1), due to the intense competition between digital development and newspapers and the production cost. In fact, 30 of newsprint mills have shut down from 2008 to 2013 in Eastern Canada and Northeastern America. Consequently, the disk refiners are idled at the site of mechanical pulping mills.

Recently, disk refining technology has attracted increasing attention as one of the mechanical pretreatment methods for lignocellulosic biomass in biorefineries [8-10]. Firstly, as a mechanical milling method, it can significantly reduce the size of particles, increase surface area and decrease the crystallinity of cellulose. Thus it has a possibility to further increase the efficiency of enzymatic hydrolysis [11]. Then, using the existing process and equipment would reduce the cost of capital investment for biorefinery plants. Thirdly, there is a great potential for commercialization of a biorefinery because the disk refining method is a highly industrialized technology. Therefore, there is a need to develop the disk refining technology for sugar extraction in a biorefinery.

1.2 Problem definition

The development of production facilities for biofuels, biochemicals and biomaterials is an essential condition for biorefineries. Currently, one challenge of sugar production from lignocellulosic biomass is to develop an efficient pretreatment process to enhance the efficiency of enzymatic hydrolysis.

Disk refining system includes a thermal process by saturated steam and a shredding process by disk refiners. It develops fiber properties for paper production by applying intense energy without chemical component removal. On the contrary, the pretreatment process requires low cost and low inhibitors (such as lignin) to reach high sugar yield by enzymatic hydrolysis. Thus, a suitable method for disk refining system must be compatible with pretreatment requirements and lead to a high-efficiency pretreatment.

Bridging the gap between pretreatment requirements and disk refining system requires that the development of a novel disk refining pretreatment would be economically feasible and ready for scale up within a short time frame. This thesis focuses on the modification of an existing disk refining system that can be directly utilized as a pretreatment process for sugar extraction.

The combination technology by chemical or by hydrothermal with disk refining pretreatment have been reported to optimize the efficiency of mechanical pretreatment [12, 13]. The operational parameters of disk refining can successfully develop fiber properties for pulp and paper making while not for sugar extraction by enzymatic hydrolysis. However, the acid methods have the disadvantages of corrosion, inhibitors and the cost of neutralization. Hot water method usually has a low sugar yield. The alkaline method is not widely effective on woody biomass. In addition, the operation of mechanical pretreatment has not applied to a pilot scale equipment yet.

Therefore, a pretreatment strategy that combines other pretreatment processes and the disk refining pretreatment were studied to improve the energy efficiency of pretreatment and the sugar yield. The operational parameters of pilot plant disk refining were further studied to increase the enzymatic digestibility and reduce the energy cost.

Moreover, impacts of this developed pretreatment process on typical lignocellulosic biomasses were further investigated. Woody biomass, agricultural wastes and herbaceous grasses were

selected as representative lignocellulosic biomass species. Then they were used to evaluate the efficiency of this pretreatment for sugar extraction by enzymatic hydrolysis.

1.3 Thesis organization

This thesis is composed of seven chapters and supported by one article. After a brief introduction in Chapter 1, Chapter 2 begins with a thorough literature review. The objectives and methodological approaches are described in Chapter 3. Chapter 4 and 5 summarizes the main results obtained in this research. The followed Chapter 6 is “General discussion”. At last, Chapter 7 shows conclusions and scientific contributions, as well as recommendations for future work.

The paper is presented in Chapter 4. It focuses on a process development of disk refining pretreatment with the title of “Validation of a novel mechanical pretreatment process for the extraction of sugars from woody biomass”. This article has been submitted to energy & fuels in January 2017.

CHAPTER 2 LITERATURE REVIEW

2.1 Lignocellulosic biomass

2.1.1 Typical lignocellulosic biomass

Major lignocellulosic biomasses are woody biomass, agricultural residues, herbaceous grasses and municipal wastes [14]. Figure 2.1 shows each species of lignocellulosic materials. In this thesis, we focus on forest biomass, agricultural residues and grasses.

- Forest biomass

Source of forest biomass includes residues left in natural forest, forestry wastes, wood chips and branches from dead trees, and cultivated forest plants [15]. In this world, 95% of woody biomass is not used every year [16]. Canada has the large and well-developed forest that occupies 41% of Canada's land area and 10% of the world's forested land [17]. It has been attracted as one of the country's important and valuable resources by the utilization of wood [17]. According to a subdivision of the division Spermatophytes (plants with seeds), wood are known as softwood (e.g. spruce, pine, and fir) and hardwood (e.g. birch, aspen and maple) [18].

- Agricultural residues

Agricultural residues are renewable, abundant and low cost resources, which are available for energy production. For example, in 2004, there are about 73.9 billion ton of dry wasted crops in the world [15]. Corn stover has an annual global production of 1413 million tons, and the price is around 83$/dry ton [19]. However, most of the residues are discarded as wastes or used as a direct fuel source (combustion). Agricultural crop residues include: field residues (e.g. straw and stalks, leaves, and seed pods) and processing residues (e.g. husks, seeds, bagasse and roots) [15]. The typical agricultural wastes are rice straw, corn stover, wheat straw and sugar bagasse.

- Herbaceous grass

Perennial grasses are an efficient and fast growing solar energy plant that is relatively easy to grow, harvest, and process. The dominant cellulosic biomasses are forages and cultivated grasses such as switchgrass, miscanthus, canary grass and alfalfa. Without competition with food sources, grasses

are considered to have energetic, economic, and environmental advantages for biofuel production [15].

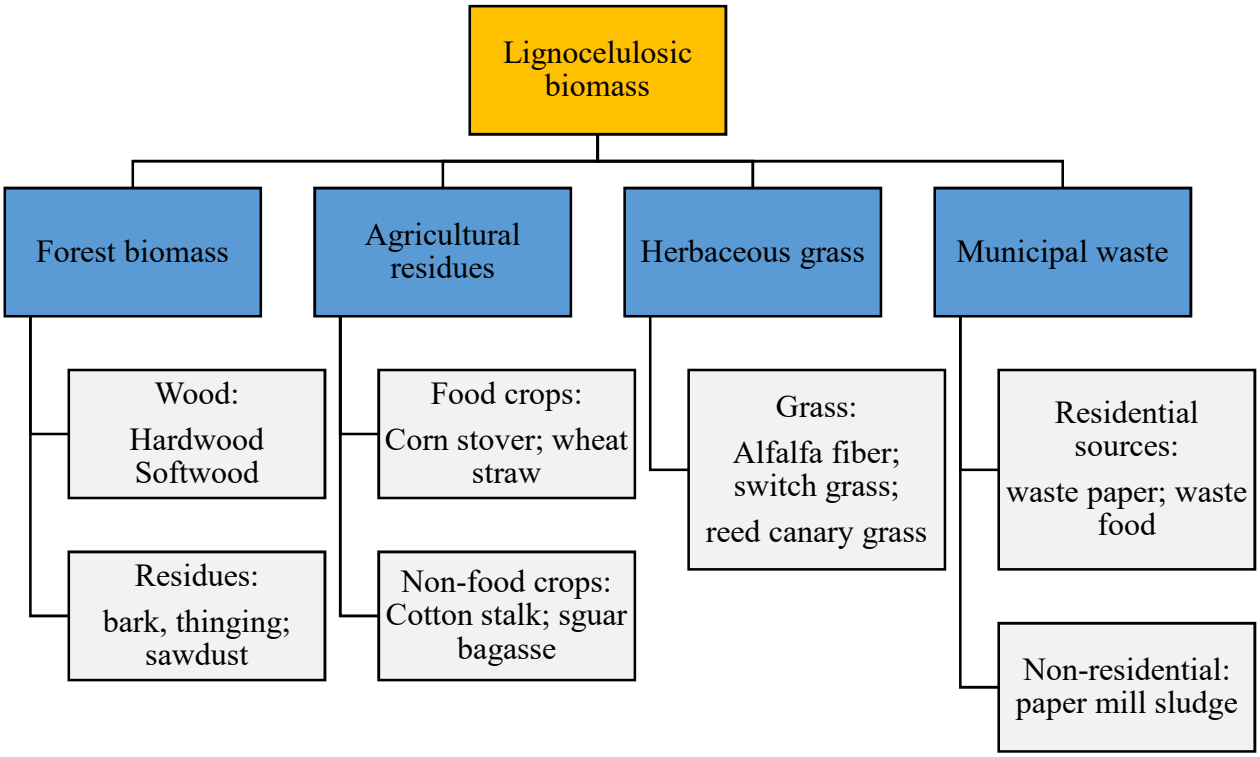

Figure 2.1 Major lignocellulosic biomasses used in biorefinery (Modified based on [14])

2.1.2 Chemical component

Lignocellulosic biomass is the most abundant organic source on the Earth, and it is the primary potential renewable and environmentally friendly resource in the world. As illustrated in Figure 2.2, it contains three major components: cellulose, hemicelluloses, and lignin.

Cellulose is a polymer of glucose linked by *β-1,4*-glycosidic bonds [20]. The chemical formula of cellulose is $(C_6H_{10}O_5)_n$ [21]. Cellulose consists of two domains: crystalline and amorphous regions. The crystalline structure is formed by a large amount of hydrogen bonds between chains due to the hydroxyl groups [22]. The degree of crystallinity of cellulose (ratio of crystallinity to amorphous domains) is one of the most important crystalline structure parameters [23]. The amount of cellulose in lignocelluloses is ~ 40-50% of the dry biomass weight [24].

Hemicelluloses include a family of polysaccharides such as arabinoxylans, glucomannans, galactans, and others that are found in the plant cell wall [21]. It is more complex than cellulose by

side group substitution. The amount of hemicelluloses in lignocelluloses is in a range from 20-40% of the dry biomass weight [24]. Softwood contains significantly more mannan and galactan. Hardwood contains appreciably more xylan and acetyl than softwood [21]. In agricultural residues and grasses, xylan is more common.

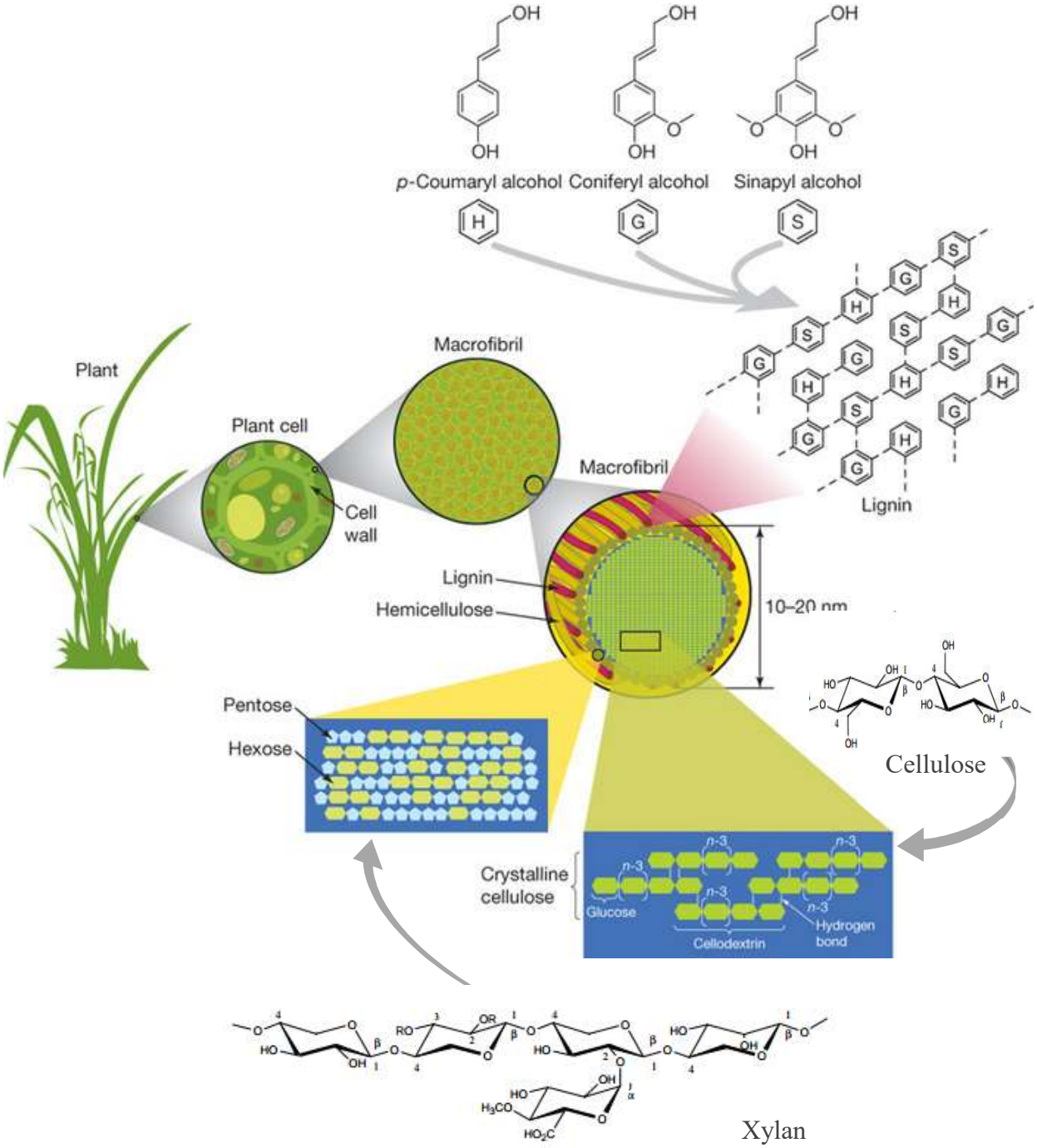

Figure 2.2: Lignocellulosic biomass structure and component (*Modified based on* [21, 25, 26])

Lignin is an amorphous three-dimensional polymer with phenylpropane units as the predominant building blocks, including p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. It is insoluble and acts as a binder of fibers [21]. The amount of lignin in lignocelluloses is ~ 10-25% of the dry biomass weight [24]. The lignin in soft wood includes confiferyl alcohol more than 90% of the total composition and the remaining being mainly p-coumaryl alcohol units [24]. In hardwood, lignin is made up of varying ratios of coniferyl and sinapyl alcohol type of units. And p-coumaryl alcohol is more prevalent in grass [24].

2.1.3 Physical structure

A plant fiber consists of different layers of the cell wall in Figure 2.3. They are middle lamella, primary wall, secondary wall (S1= outer layer of secondary wall, S2= the secondary layer, S3= inner layer of secondary wall) and warty layer [27]. The most content of cellulose is contained in the S2 wall, while a high concentration of lignin is in middle lamella [28]. However, more than 70% of total lignin located in S2 because of its thickness.

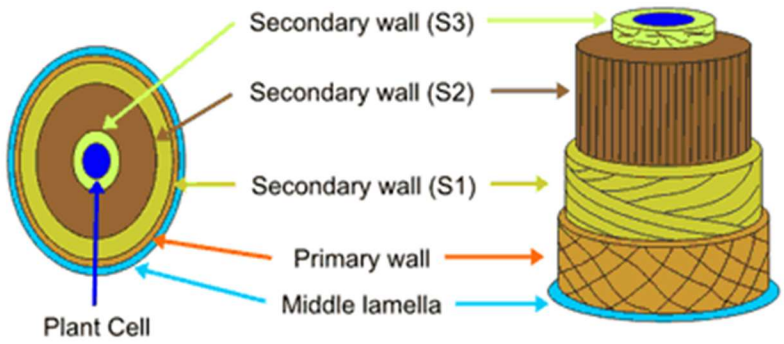

Figure 2.3: Schematic representation of the cell wall layers [27]

2.2 Biochemical conversion process

Since the additional fossil-derived carbon dioxide is a burden to the environment, alternative methods of reducing the greenhouse gas (GHG) emission have to be explored [16, 29]. The biorefinery is a concept of using the renewable biomass as raw materials to produce various high-value products fuels, power, and chemicals [3, 30, 31]. Biochemical conversion pathway is the most favorable method to extract fermentable sugars by enzymes according to the advantages of high selectivity, environmentally friendly, and potential to achieve the theoretical yield of sugar

[24]. Unlike the sucrose or starch based on biofuels or chemicals production, lignocellulosic biomass requires an advanced conversion process shown in Figure 2.4.

Pretreatment is a processing step to break down the lignocellulosic biomass structure and provide an accessible substrate for enzymes attack [5, 24]. Enzymatic hydrolysis is being performed by addition of adequate amount of enzymes into the pretreated biomass to depolymerize carbohydrate polymers to produce fermentable sugars [24]. The fermentable sugars are further metabolized by yeasts to bioethanol or other chemicals in the step of fermentation. The distillation is to purify the products to meet their specifications.

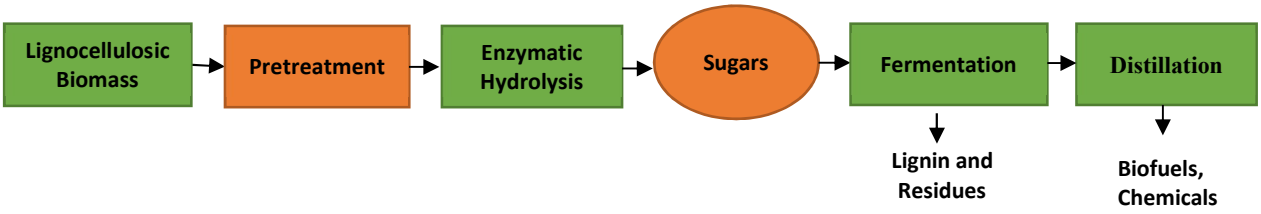

Figure 2.4: Typical biochemical process

2.3 Biomass pretreatment

It is well known that the nature wood biomass has the recalcitrant to limited the hydrolysis efficiency. In substrate-related factors, lignin reduces the efficiency of hydrolysis because it inhibits enzyme accessibility; the crystallinity of cellulose is one of the main reasons for limiting the enzymatic hydrolysis [21]; the degree of polymerization, hemicellulose content, the feedstock particle size, pore volume, specific surface area, and acetyl group content are also the factors [24].

2.3.1 Key factors for an effective pretreatment of lignocellulosic biomass

The principle of a pretreatment process is to break down the lignin structure and to increase the available surface area for enzymes attack (Figure 2.5) [32]. An effective pretreatment would have four features [32, 33]:

1) More carbohydrates recovery. The pretreatment needs a high recovery rate of all carbohydrates. It means the pretreatment yield of fermentable cellulosic, and hemicellulosic sugars are close to 100%. Thus the pretreatment process requires no degradation of hemicelluloses and cellulose.

2) High digestibility. Over 90% digestible of cellulose from pretreatment is required at a hydrolysis conditions of less than 72 hours (maximum 96 hours) with enzyme loading lower than 10 FPU/g cellulose [21].
3) Low cost pretreatment. It includes two parts:
   - The pretreatment process has both low capital and operational cost.
   - This process doesn't cause the cost increase in downstream such as detoxification and high enzymes usages.
4) Low content of inhibitors. The pretreatment of lignocellulosic biomass has negative effects on hydrolysis and fermentation by the inhibitors from degradation of hemicellulose or lignin. For example, carbohydrate can be degraded to inhibitors such as furan aldehydes and aliphatic acids. The inhibitors of phenolics are mainly from lignin. This requires the pretreatment process avoiding the formation of byproducts inhibitory to the hydrolysis and fermentation process.

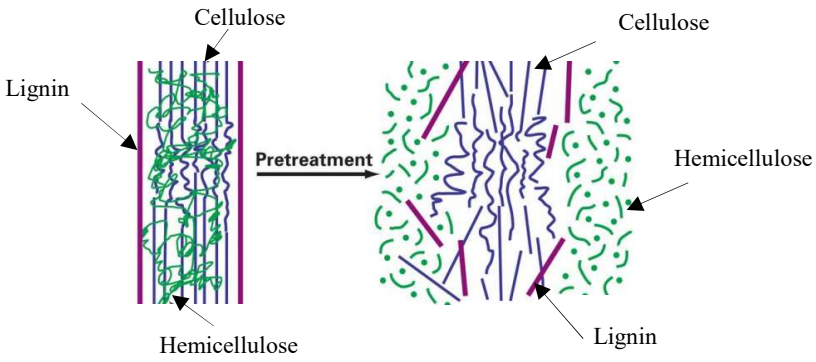

Figure 2.5: Schematic of pretreatment (modified based on [32])

2.3.2 Pretreatment technologies

In general, there are two types for pretreatment methods: fractionation and delignification [19]. Fractionation is to improve access to polysaccharides by disrupting biopolymer matrix; while the delignification is to remove lignin [19].

- **Biological pretreatment**: It employs fungi to break lignin molecular such as white-rot and soft-rot. This is an expensive pretreatment method because of the high cost of microorganisms and long reaction time [19].
- **Chemical pretreatment**: Utilization chemical substances to fractionate lignocellulose is widely known as pretreatment methods. Higher glucose yield can be obtained by removing

hemicellulose or lignin. Typical chemicals that are commonly used are acid, alkaline, ionic liquids, organic solvent and surfactant [19].

- **Physical pretreatment**: Mechanical method and microwave irradiation are the two most common methods in physical pretreatment; The mechanical method also name as size reduction. The irradiation method is very easy for operation and has high heating efficiency.
- **Combination pretreatment methods**: The combination methods usually include thermo-physical and thermochemical pretreatment. One type of thermochemical methods uses water/steam as the solvent. For example, liquid hot water (LHW) at high temperature and pressure to hydrolyze biomass. Steam explosion uses high temperature and pressurized steam with a fast pressure release. Another type of thermochemical pretreatment is ammonia-based biomass pretreatment, such as ammonia fiber/freeze explosion (AFEX). Supercritical is a thermos-physical pretreatment, which is similar to steam explosion.

Each pretreatment has different impacts on compositional and structural feathers of biomass. Table 2.1 shows the mode, advantages and disadvantages of some typical pretreatment methods.

- **Acid pretreatment**

Acid pretreatment hydrolyzes hemicelluloses and releases monomeric sugars and soluble oligomers from the cell wall matrix into the hydrolysate. The enzymatic digestibility can be improved by hemicellulose removal [34]. The maximum enzymatic digestibility usually coincides with complete hemicellulose removal [34]. The typical acid includes H_2SO_4, HCL and peracetic acid [19].

- **Alkaline pretreatment**

The major effect of alkaline pretreatment is to remove lignin from the biomass, thus improving the reactivity of the remaining polysaccharides. It also minimizes the deactivation of enzymes due to their binding to lignin, which can improve the enzymatic hydrolysis of cellulose [35]. During an alkaline pretreatment, the first action is solvation and saponification. Moreover, alkaline pretreatment have additional effects on the removal of silica (acid-insoluble component from ash), or on the partial removal of hemicelluloses (including acetyl groups and uronic acids) and cellulose swelling, resulting in a substantial increase of the fiber surface area [35]. The removal and modification of lignin have an effect on energy consumption as well. The common chemcials are

lime (calcium hydroxide) or sodium hydroxide. This pretreatment is carried out at a room temperature or a higher temperature (20-121˚C), during the time ranging from minutes to hours (15-121mins), and alkaline concentration from 0.5% to 8% w/v [35].

- **Hydrothermal pretreatment**

Hydrothermal pretreatment is also named as autohydrolysis technology. It is using water/steam to treat biomass at high temperature and pressure. The dissolve of hemicellulose is from the acid released from cellulose/hemicellulose [21]. It has several attractive features as a pretreatment method including [21]: Water is the only medium; No chemical added; Environmental friendly; No chemical recovery cost; Less corrosion caused by acid. The key factors of steaming are treatment time (t, time), temperature (T, °C) and particle size [36].

- **Mechanical pretreatment**

Mechanical pretreatment is one of the physical pretreatment methods. It increases the specific surface area of biomass and reduces the degree of polymerization and cellulose crystallinity [19]. Several well-developed technologies are available for biomass size reduction, such as hammer milling, knife milling, shredding, and attrition milling. Traditionally, the mechanical pretreatment methods are reported as not economically feasible technologies. Because their energy consumption is higher than the theoretical energy content available in the biomass in most cases [32]. Therefore, limited research efforts and resources have been devoted to these critical areas [3, 8].

In fact, there are many advantages by mechanical pretreatment.

(1) It increases the volume of calorific value of biomass and simplifies the densification processes [37];

(2) It simplifies the supply chain of raw materials, and their storage conditions [37];

(3) It increases the total accessible surface area, thus it improves the bio-accessibility of constituents and the conversion of saccharides during hydrolysis [11, 37];

(4) It reduces the mass and heat transfer limitations during the hydrolysis reactions and consequently reduces energy inputs [37].

Table 2.1: Advantages and disadvantages of different pretreatment methods for lignocellulosic biomass [19]

Pretreatment	Mode	Advantages	Disadvantages
Mechanical	- Reduces cellulose crystallinity - Increase surface area - fiber size reduction	- Simple operation - Handle large volumes of biomass - No use of chemicals - Very little inhibitors generated	- Low sugar yield - High energy consumption
Dilute acid	- hydrolyzes hemicellulose to xylose and other sugars; - alters lignin structure	- Dissolution of hemicellulose - High sugar yield	- High costs of acids and need for neutralization - Corrosive resistant equipment is required - Formation of inhibitors
Alkaline	- Removes hemicelluloses and lignin; - Increases accessible surface area	- Low reaction temperature	- long residence times required; - Irrecoverable salts formed and incorporated into biomass
Autohydrolysis	- Causes hemicellulose degradation and lignin transformation; - Cost-effective	- No corrosion equipment required - Suitable for hardwood	- Formation of inhibitors - Requires to remove inhibitors - Incomplete disruption of the lignin-carbohydrate matrix; - Generation of compounds inhibitory to microorganisms
AFEX	- Increases accessible surface area - Removes lignin and hemicellulose to an extent	- Effective for agricultural wastes - High sugar yield - Low formation of inhibitors	- Recycling ammonia is expensive - Hemicellulose is not hydrolyzed - not efficient for biomass with high lignin content
Biological	- Degrade lignin	- Low energy requirements	- Long treatment time - Low enzymatic hydrolysis rate

2.4 Disk refining pretreatment technology

2.4.1 Disk refining system

The TMP process was developed based on RMP in the 1970's [38]. In a typical TMP mill, it mainly includes steaming, refining and latency steps (Figure 2.6).

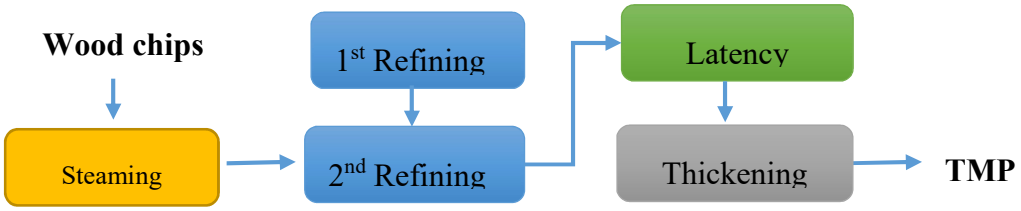

Figure 2.6: The typical TMP process [39]

- Steaming

The wood chips after washing firstly go through steaming vessel around 1-10 min with saturated steam [18]. Softening of the wood chips before refining is usually beneficial to keep the fiber lengths intact and to achieve flexible fibers from the process.

- Refining

TMP is generally carried out in two stages of refining. Preheated wood chips are fed into first refiner (primary refiner) where the chips break into fiber bundles and separate into single fibers at elevated temperature (110-130 °C) and pressure [6]. Similar to the steaming stage, the higher temperature during the first refiner softens the fibers, which keeps the maximum of fiber length and produces the minimum content of fines. The refining temperature is just under the glass transition temperature[1] of lignin (approximately 140 °C), which avoids the soften lignin coating on fibers. In the second stage, the refiners are at ambient temperature to treat the fibers for papermaking by improving fibrillation (surface area) and increasing access to hydroxyl groups for hydrogen bonding [18].

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[1] Glass transition temperature is referred to the temperature where amorphous polymers changes its physical characteristics by heating resulting in transitions to a glassy and rubbery state [40] L. H. Sperling, *Introduction to physical polymer science*: John Wiley & Sons, 2015..

- Latency removal

A latency removal step is after the refining step [39]. Latency refers to a high curl developed in fibers during refining which is frozen upon cooling after refining.

2.4.2 Refiners

A refiner consists, in essence, of two patterned discs: single disk refiner and multiple disk refiners [23]. Figure 2.7 shows the structure of a single disk refiner. It constituted by one rotator plate, one stator plate and a motor. The rotor and stator could have various patterns of plates with different grooves and bar dimensions.

These plates are separated into different sections in Figure 2.8 [23, 38]. Zone 1 is the most inner part of the refining, which is referred as breaking zone. Zone II is the middle part of the refiner as intermediate refining zone. And Zone III is the narrow disc gap as outer refining zone. Wood chips carried with water go through breaking zone: the 1st section (inlet) rips chips into wood shives. And each subsequent step reduces the size of the bundles [38].

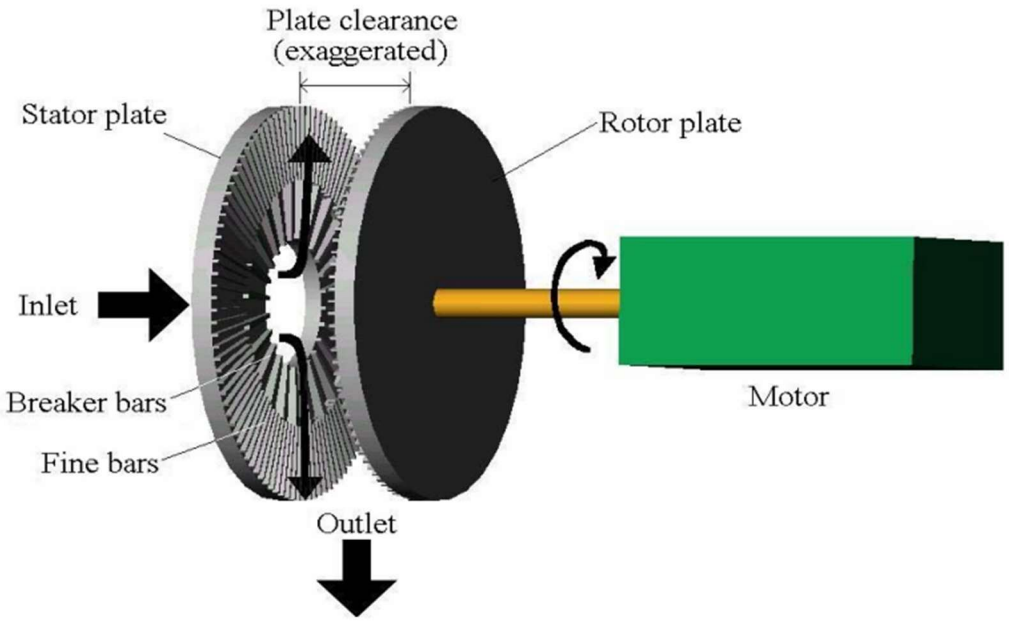

Figure 2.7: Cross-section of single-disk refiner [41]

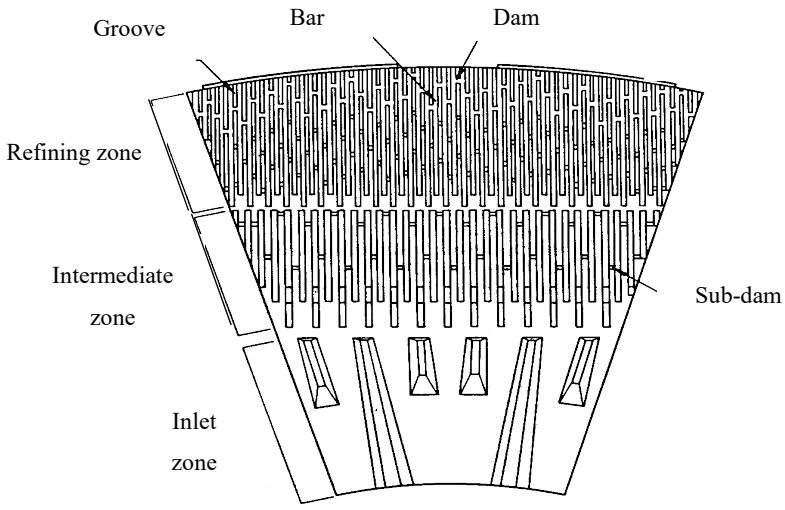

Figure 2.8: Segment of disk refiner (modified based on [38])

2.4.3 Refining mechanism

A generally accepted theory about refining mechanism is that fibers are subjected to repeated compressions and decompressions from bar crossing. The development of fibers occurs because of the process of wood fatigue [38]. The forces which are transmitted to the flocs are normal force, shear force, and corner or edge force (Figure 2.9) [23]. The normal force is due to the compression of the floc between bars. Shear force is produced by the movement of bar surface on the floc. Fibers in contact with other fibers are also possible to have shear forces. The corner or edge force appears at the bar edge [23].

There are three dominant refining stages (Figure 2.10) [23].

- Stage 1: Pick-up stage. Fibers are accumulated and received the first hit from edge to edge on the bars of the plates. The fibers also get compression from the edge and the surface of the plates.
- Stage 2: Sliding stage. The fibers are pressed between stator and rotor. The trapped fibers are compressed by the surfaces of the moving and stationary bars.
- Stage 3: Releasing stage. The fibers are affected by shear forces. In this stage, the fibers also hit the bars on the surface to edge and again edge to edge. During the bar crossing, two different forces act on the fibers, one of them due to the contact fibers to bars and another one due to the contact fiber to fiber.

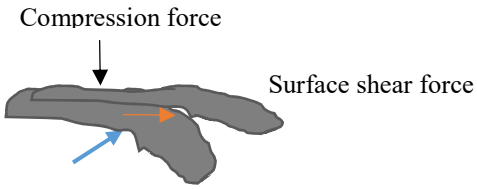

Figure 2.9: Forces transformed in fibers (modified based on *[23, 42]*)

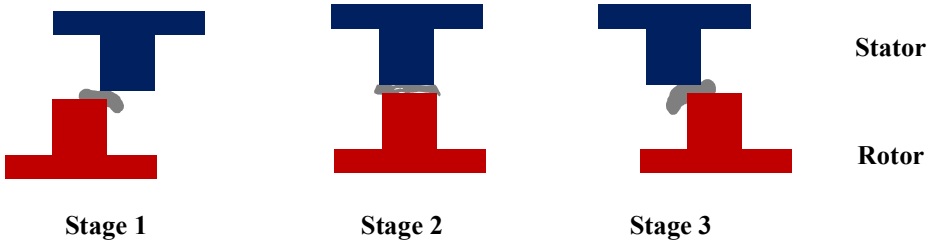

Figure 2.10: Schematic three dominant refining stages (Modified based on [23])

2.4.4 Structural properties of fiber during refining

Due to refining is a complicated and simultaneous process, the exact mechanism of fiber refining is not understood yet. An ideal refining mechanism through which the development of refining occurs is showed in Figure 2.11 [28, 38]. It mainly includes two steps: separation and fibrillation. The separation step is the disintegration of particles, which means to break down the initial chips into shives and fiber development by disintegration to single fibers. Then fibrillation step includes two pathways. One path is cutting long fiber to short fiber thus generate fines. Another path is a peeling mechanism which generating short fibers, fines, and more flexible long fibers.

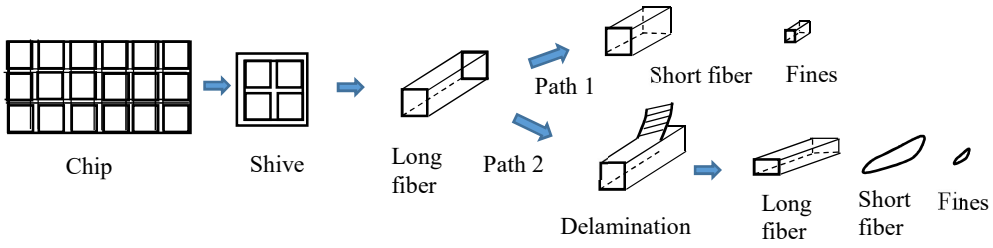

Figure 2.11: Separation and development of fibers in refining (modified based on [38])

The major effects of refining of fibers that result in changes in fiber structure are categorized into six groups: internal fibrillation, external fibrillation, fines formation, fiber shortening, crystallinity and redistribution of surface chemical compositions.

- Internal fibrillation

Internal fibrillation occurs from delamination of the P and S1 layers, caused by the cyclic compression action of forces inside the refiner [23]. The inner bonds are between cellulosic fibrils, between fibrils and hemicellulose, between cellulose and lignin, and between hemicellulose and lignin. The breakage of inner bonds causes the pore structure inside the cell wall to expand and swelling. Actually, swelling is an evidence of internal fibrillation during refining [43]. Also, fiber is more flexible after internal fibrillation due to the loosening of the fiber wall or reducing the bending stiffness of the fiber wall [23].

- External fibrillation

It is peeling off the fibrils from the fiber surface and exposing the S2 layer [23]. It is induced by the impact of refiner bars, bar-fiber, and fiber-fiber from mechanical stress [44]. These fibrils usually have large surface area.

- Fines formation

Fines formation is a result of external fibrillation by excessive force [23]. The fines defined as loosening fibrous material are less than 0.3 mm which significantly increase the specific surface area of fibrils [44]. The percentage of fines is increased by refining time or shear rate [23].

- Size reduction

The reduction of raw material size is achieved using a combination of different mechanical stresses such as impact, compression, friction, and shear [37]. Fibers break when the strain on a fiber is high enough. Accordingly, the direct shearing forces of the passing refiner bars can cut fibers. Or the fiber breakage happens when pulled from a network of other fibers [23].

- Crystallinity

The swelling is affected by crystallinity. A decreased crystallinity makes a higher absorption of water of the cell wall [23]. Chen, et al. [45] reported that crystallinity of cellulose increases first then decreases during the refining due to the forces. And it was reported that the high degree of crystallinity of nanofibrillated fiber can be reduced by several steps' refining [23].

- Chemical composition

It is reported that chemical composition can be released and exposed during refining because of the outer surface stripped off and new layers generated [46]. Also, the dissolved materials may reprecipitate on the surfaces. More research shows the functional groups slight variation occurs in the distribution of surface chemical compositions [23]. For example, during mechanical defibration, wood is subjected to high temperatures up to 180°C (above the lignin glass transition temperature) and diluted to low consistencies (2-4%) with hot water (60-80°C) [27]. The results show volatile compounds evaporate off; some water-soluble sugars dissolve. For extractives, they are dispersed or even dissolved but may also be redeposited. Part of the middle lamella lignin may be removed and dispersed.

2.4.5 Effects of disk refined fiber structure on enzymatic hydrolysis

Through refining, fiber structure changes in terms of size (length and thickness), surface area, pore volume, crystallinity, and chemistry. Table 2.2 summarizes the relationship between biomass features and enzymatic digestibility of cellulosic biomass.

- Particle size

Size reduction has been reported to increase reaction rates of hydrolysis, increase the conversions of cellulose to glucose, and reduce the viscosity [11, 37]. For example, a reduction in particle size from 590-33 μm resulted in a 55% increase in glucose production after 72 h cellulose hydrolysis [37]. The rate can be doubled in a 10 h reaction experiment when the average size of cellulose is reduced from 82 to 38 μm [37]. Particle sizes reduction possibly affects its molecular structure to facilitate enzymatic accessibility. It could enhance the affinity between cellulose polymers and enzymes and thus increase the rate of hydrolysis[37]. However, it was also reported the different size fraction (1.42-0.21mm) of milled corn stover almost had a continually increased yield of enzymatic hydrolysis (approximately around 24-27%) [47].

Cadoche and López [48] studied the relationship between size reduction of hardwoods and agricultural wastes and final particle size and ratio of comminution, and they found that the energy input and final particle size are depending on each combination of raw material and equipment.

- Specific surface area

The available surface area includes the external and internal pore structures, which is determined by many of the features mentioned. The external surface means they are visible under light microscopy. It includes the gross surface of the fiber, the lumen, and the pit-aperture and pit-membrane pores. Then, those spaces between the microfibrils and the ends between the crystallites are internal surface. The total external surface area of cellulose fibers is generally on the magnitude of 1 m^2/g [49]. However, the internal surface area is much larger than an external surface area with a range from 300 to 600 m^2/g for sufficiently swelled fibers [49]. Therefore, the accessibility of pore size is vital for enzymes to intact with the internal surface area.

The substrate surface area is most relevant to heat and mass transfer, enzyme accessibility, and energy consumption. It is general accepted that hydrolysis yield increases with a higher specific surface area [47, 50]. For example, small particles sizes of the untreated cellulosic substrate are more readily hydrolyzed as compared to larger ones. Because the high specific surface area of substrates can offer better interactions between enzymes and substrates. These hypotheses were confirmed by using ground corn stover in size ranges of 425-710 and 53-75 μm [51]. It is stated that the energy for size reduction was proportional to the newly created surface area [52].

- Fiber wall pores volume

Pore size 51 Å is considered being equivalent to the enzyme molecular size [53]. Thus the accessibility of enzymes to pore structure is limited because the pore size of the capillaries is mainly smaller than 3 nm although it can be around 20 nm. Thus, the accessible surface of the pore structure is of particular importance when evaluating the physical structure and substrate digestibility [49]. During the disk refining, fiber walls are separated. Consequently, a previously inaccessible pore volume may be revealed. During the internal fibrillation, the breaking bonds within the cell wall can also increase the pore volume accessible to cellulase enzymes.

- Crystallinity and chemistry of the fiber wall

The crystallinity of lignocellulosic feedstocks has been shown to be a major determinant of enzymatic saccharification efficiency [22]. An entirely amorphous sample hydrolyzes much faster than a crystalline cellulose.

Chemistry of fiber wall plays critical role in hydrolysis yield, and it has been wildly studied. Generally, lignin removal and hemicellulose removal improves cellulose accessibility. However, it was also reported that removal of hemicelluloses is more critical than delignification in improving substrate cellulose accessibility because hemicelluloses are more closely associated cellulose [53]. The similar report showed hemicellulose removal is more important than lignin removal of creating an accessible process of cellulase [54].

Table 2.2: Correlations between biomass features and enzymatic efficiency

Biomass features	Refining mode	Enzymatic digestibility	Reference
Specific surface area	External fibrillation, fines formation, fiber shortening	Positive	[47, 55]
Swelling	External fibrillation	Positive	[56]
Cellulose crystallinity	Sensitive to degree of refining and refining conditions	Negative	[55]
Final particle size	Fiber shortening External fibrillation	Negative with limitation	[22, 47, 49, 54]
Pore volume	Internal fibrillation	Positive	[49]
Lignin	Temperature	Negative	[54, 55]
Hemicellulose	Temperature	Negative	[55]

2.4.6 Refining energy consumption

Generally speaking, compared to non-refining pretreatment, the disk refining pretreatment (DRP) is an efficient pretreatment method with an improved the sugar yield significantly [57]. However, using the disk refiner is energy intensive to reach the required fiber structure. For example, it was calculated that energy consumption of size reduction account for about 10-40% of the thermal energy of ethanol produced from wood [11]. The problem of saving energy has been much researched over the years in paper making [28].

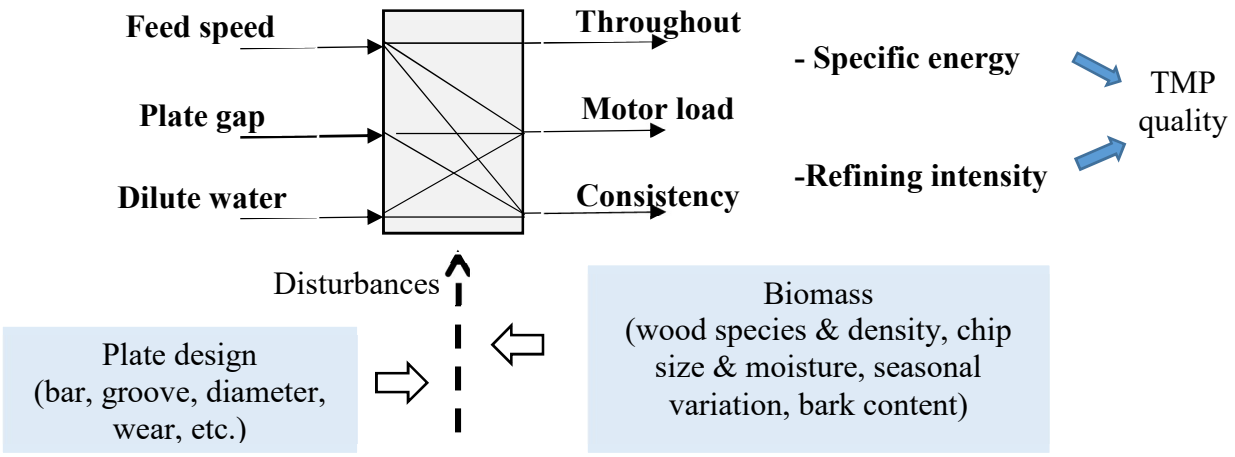

Figure 2.12: Control strategies of a refiner (Modified based on [58])

Typical controlled and manipulated variables in refining are illustrated in Figure 2.12 [58]. For obtaining a uniform pulp quality, feed speed, plate position, and dilution flow are used to control the three output variables of throughput, motor load, and refining consistency. Wearing of refiner plates and variations in raw materials are the two principal types of disturbances in a refining system. Refining is a multivariable system, its fiber quality is a function of two variable: specific energy and refining intensity [58].

- Net specific energy

One parameter that contributes to the study of different theories is specific refining energy or net specific energy (NSE). It is used for calculating how much energy is given from the refiner to the fibers, and it can be obtained by dividing the net power by the fiber mass (flow rate) (Equation 2.1) [23, 59]. The higher NSE, the more refining is happening to the fibers [58].

$$NSE=\frac{P_{net}}{\dot{M}}=\frac{P_{tot}-P_{no-load}}{\dot{M}} \qquad \text{Equation 2.1}$$

Where, P_{tot} =the total power consumed (kW); P_{net} = the net power (kW) consumed to change the pulp properties; $P_{no\text{-}load}$ =the initial power or no-load power (kW) which is defined as the power needed to rotate the rotor in the refiner; $\dot{M}$ =dry weight of refined pulp per hour (ton/h).

According to the concept of NSE, it is used for calculating how much energy is given from the refiner to the fibers [23, 59]. The higher NSE, the more refining is happening to the fibers. Based on the fiber structural changes during refining, the energy applied to fibers is consumed on two

stages during refining: defibration (separation) and additional fiber modification (fibrillation, collapse, and others).

$$E_{net} = E_d + E_f \quad \text{Equation 2.2}$$

Where, E_{net} is the energy applied to fibers, E_d is the energy consumed in the defibration stage; E_f is the energy consumed in the additional fiber modification stage including fibrillation, collapse and others. It has been reported that the E_f is much higher than $the\ E_d$.

In TMP mills, the factors of influence of refining are from the materials, the refiner plates, and the operation conditions. The ideas for energy savings have usually from the following concerns: (1) material pretreatment, such as heat, chemicals, enzymes or fungi; (2) modification of refiners, for example new segments design; (3) better operation conditions, such as temperature, consistency, plate gap, rotation speed [28, 38].

- Refining intensity

The refining intensity describes how rapid energy is put into pulp, and it is also called the intensity of each impact. It is a function of cumulative forces applied on the fiber during its residence time in the refiner. The relationship between I and NSE is shown in Equation 2.3 [23, 59].

$$NSE = N \times I \quad \text{Equation 2.3}$$

Where N is the number of impacts; I is the intensity.

Intensity is a function of cumulative forces applied on the fiber during its residence time in the refiner. Equation 2.4 [23, 59] describes how the refining intensity can be altered by changing the power input, rotational speed, number of segment bars, and pulp consistency that would affect t_r.

$$I = \frac{P*60}{n*n_b*t_r} \quad \text{Equation 2.4}$$

Where I is the intensity, P_{tot} is the power consumption, n is the rotational speed (rpm), n_b is the number of bars, t_r is the residence time.

Because N and I can be considered as the split in Equation 2.5 and 2.6 [23, 60]. It is also called C-factor theory.

$$N = \frac{C}{\dot{M}} \quad \text{Equation 2.5}$$

$$I = \frac{P_{net}}{C}$$ Equation 2.6

Where C is a function of fiber length, coarseness (mass per unit length), plate geometry, rotation speed and pulp consistency.

According to the equation of the C-factor theory, the key parameters affect refining intensity are specific energy, refining pressure/temperature, production rate, consistency, rotational speed, plate pattern.

2.5 Disk refining pretreatment: operation parameters and combination technologies

2.5.1 Refining parameters

a. Refining consistency

The refining consistency of refining process significant influences refining energy consumption [61]. For example, reducing the consistency from 50% to 3% saved about 20-80% energy [61]. However, in the TMP refining system, the typical solid loading is 10-20%. The mechanism of refining consistency on hydrolysis efficiency is not clear yet. High consistency refining causes fewer fines formation while low consistency refining tends to produce more fines [23]. Zhu et al. reported that low consistency slightly changed the efficiency of enzymatic hydrolysis of softwood.

b. Disk plate gap

Refining disk plate gap is another refining process parameter that can be adjusted to minimize energy consumption. Increasing disk plate gap can save 80% to 90% energy when the gap increases from 0.38-2.54 mm [61]. But, due to the specific surface has a linear inverse relationship with disk plate gap, the enzyme hydrolysis yield is decreased at a larger gap. Zhu et.al. considered 0.76 mm as the refining gap [61]. This gap size is higher than the critical gap (around 0.30 mm), thus the fiber size is still large.

c. Temperature

During refining, lignin has a low glass transition temperature (120-140°C), the refining temperature over 140 °C will cause lignin coating on the substrates [61]. It understands that at a low temperature of 140 °C, wood chips can be fractured in the S1 and S2 layer of the cell wall. However, when the

steam pressure up to 166 °C (7.2 bars), wood chips are fractured in the lignin-rich middle lamella (ML). This probably results in a low enzymatic digestibility. In the steaming digester, a high temperature and pressure is to soften wood chips for reducing the energy consumption of refining.

2.5.2 Combination technologies

In the refining process, separation of fibers occurs in the weakest part of the wood, which depends on moisture, temperature, and frequency, but pretreatment methods (chemical, mechanical, and biological) could also be possible factors. Chemical pretreatment can change the chemical composition and physical structure of wood by partially removing some cell wall components such as hemicellulose and lignin [61]. Table 2.3 compared the literature data of the combination pretreatment of hot water, acid, alkaline, or autohydrolysis before DRP of different biomasses.

2.5.2.1 Dilute Acid

Zhu et al. reported sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL). It consists by sulfite treatment of wood chips under acidic conditions and mechanical size reduction using disk refining [62]. In SPORL, sulfuric acid and bisulfite are applied for wood chips to degrade hemicellulose. The removal of hemicellulose leaves pores in wood structure, which weaken the wood matrix and thereby reduces the energy consumption for size reduction [61]. Sulfite pretreatment can sulfonate lignin to reduce the negative effect of enzymatic hydrolysis [63].The sulfonation makes lignin hydrophilic, which promots swelling and softening of wood chips and consequently decreased energy consumption for size-reduction [61]. Then partial sulfonation of lignin during low pH SPORL with acid pretreatment is another reason for its low energy consumption for size-reduction [10].

2.5.2.2 Alkaline pretreatment

Chen et al. [13] used NaOH combined DRP to achieve high hydrolysis efficiency of corn stover at a low temperature (80 °C) for 2 hours with alkaline (40 kg NaOH /ODMT corn stover) under refining energy (128-468 kWh/ODMT). They found that, compared to pretreated corn stover controls, the deacetylated corn stover feedstock is approximately 20% more digestible after pretreatment [13]. The deacteylated biomass reached high yields of xylose and glucose by enzyamtic hydrolysis. Moreover, this process can reduce toxics produced during pretreatment [13].

2.5.2.3 Hydrothermal

It was reported that hot water combined with DRP has much lower hydrolysis yield and lower energy consumption than dilute acid DRP [64]. However, Han et al. [24] reported the autohydrolysis can significantly improve the enzymatic digestibility for agriculture wastes. They studied the wheat straw, switchgrass, corn stover, waste wheat by autohydrolysis (180°C, 10 min) combined a disk refiner plus PFI disk refiner (a lab refiner) strategy for corn stover at 10% consistency, 6000 revolutions. The disk refiner is aimed at disintegrating the fiber, while the PFI post-treatment is to create microfibrils and to loosen internal fiber structure [24]. They found that the mechanical refining contributed to the improvement of enzymatic sugar yield by as much as 30%.

Table 2.3: The total sugar recovery using DRP methods for different biomasses

Wood species	Biomass	Chemical treatment	Chemicals (w/w biomass)	Chemical treatment Conditions	Refining condition	Energy (kWh/ton)	ECSS (%)	Ref.
Softwood	Pine	None	-	-	2570 rpm	616	11.3	[61]
	Pine	Autohy.	Hot water	180°C, 30 min	2570 rpm	537	33.1	[61]
	Pine	Acid	$NaHSO_3$ 8%	180°C, 30 min	2570 rpm	499	84.1	[61]
Hardwood	Aspen	Acid	H_2SO_4 2.21%, $NaHSO_3$ 8%	180°C, 30 min	0.25 mm	-	96	[65]
	-	Alkaline	6.5% Na_2CO_3	-	-	698	46.23	[66]
Grass	Alfalfa	Autohy.	Water	-	-	-	59-65	[67] a
	Switch-grass	Autohy.	Water	190°C, 20 min	PFI 6000 rev	-	75.9	[24] a
Agri. residues	Corn stover	Autohy.	-	180°C, 10 min	0.127 mm	-	70.6	[24]
	Corn stover	Acid	H_2SO_4 0.5-1.4%	165-195 °C, 3-12min	-	-	80-87% Cellulose	[68]
	Corn stover	Alkaline	NaOH 8%	80°C, 2h	-	128-468kwh/odmt	65-84	[13]

Note: ECSS: enzymatic cellulose conversion of substrate, %; a: reducing sugar yield; Ref: reference; Agri. residues: agricultural residues; autohy.:autohydrolysis; PFI: a laboratory pulp beating mill

2.6 Critical review

There have been several suggestions on approaches to apply a disk refining system as a pretreatment technology to enhance the efficiency of enzymatic hydrolysis and reducing energy consumption.

According to the operation conditions of disk refining, the parameters of gap and consistency have not been widely studied for the pretreatment.

The combination technologies have advantages and disadvantages depending on the chemicals used. The acid pretreatment before DRP offers excellent performance for sugar recovery, but there are also some drawbacks. The hemicellulose sugars might be further degraded to furfural and hydroxymethyl, strong inhibitors to microbial fermentation. Furthermore, acids can be corrosive and neutralization results in the formation of solid waste. It is not very favorable of high lignin content biomass [10]. Besides, the drawbacks of using chemicals are the recovery of chemicals and remove the toxics. The autohydrolysis could avoid the acid effects on environment and equipment. However, the high temperature of reaction may cause the byproducts generation including acetic acid, formic acid, furfural, and HMF. For example, acetic acid produced by hydrolysis of acetyl groups from hemicellulose is the major byproducts generated during autohydrolysis [24]. When the temperature is higher than 190 °C, furfural starts to form in a significant amount due to the degradation of pentose [24]. For alkaline pretreatment before DRP, it is very suitable for agricultural residues. However, the reaction time is usually too long.

Besides, the lack of research of energy consumption of the disk refining pretreatment is very common. The most of research are conducted in labs and only focus on one species of biomasses.

Based on the literate review, the following areas were identified to require further investigation:

(1) The enzyme hydrolysis yield of the thermal treatment on disk refining is not typically evaluated.
(2) Only few studies involve in the alkaline and thermal treatment to increase the efficiency of disk refiner system.
(3) There is limited knowledge of operation conditions of a pilot scale disk refining system as a pretreatment method on efficiency of energy consumption and enzymatic hydrolysis.
(4) The effects of biomass species on enzymatic hydrolysis are rarely considered in proposed disk refining pretreatment processes.

CHAPTER 3 OBJECTIVES AND METHODOLOGY

3.1 Objectives

The literature review shows current work on a broad range of pretreatment methods for enhancing efficiency of enzymatic hydrolysis from lignocellulosic, the fundamental of disk refining, as well as current research status of disk refining pretreatment. Several chemical or hydrothermal pretreatment methods were developed, the main application of the well-known disk refining pretreatment have been studied in a laboratory scale. However, significant scientific and technological breakthroughs are necessary to enhance industrial applications and commercialization.

Hence, the mian objective of this work is:

> modifying the existing disk refining system to obtain a mechanical pretreatment process with a high sugar yield of enzymatic hydrolysis and a high energy efficiency of refining for different lignocellulosic biomasses.

The sub-objectives are:

(1) To develop a high-efficiency disk refining pretreatment to improve sugar yield of enzymatic hydrolysis.

(2) To investigate the performance of the developed pretreatment technology on different lignocellulosic biomass species.

3.2 Methodology

The disk refining system used in this study is shown in Figures 3.1. It is pilot plant scale and located at Centre for Research on Lignocellulosic Materials, Université du Québec à Trois-Rivières, QC, Canada. Figures 3.2 illustrates its schematic of the pilot plant disk refining system.

Figure 3.1: The pilot plant disk refining system in the Université du Québec à Trois-Rivières

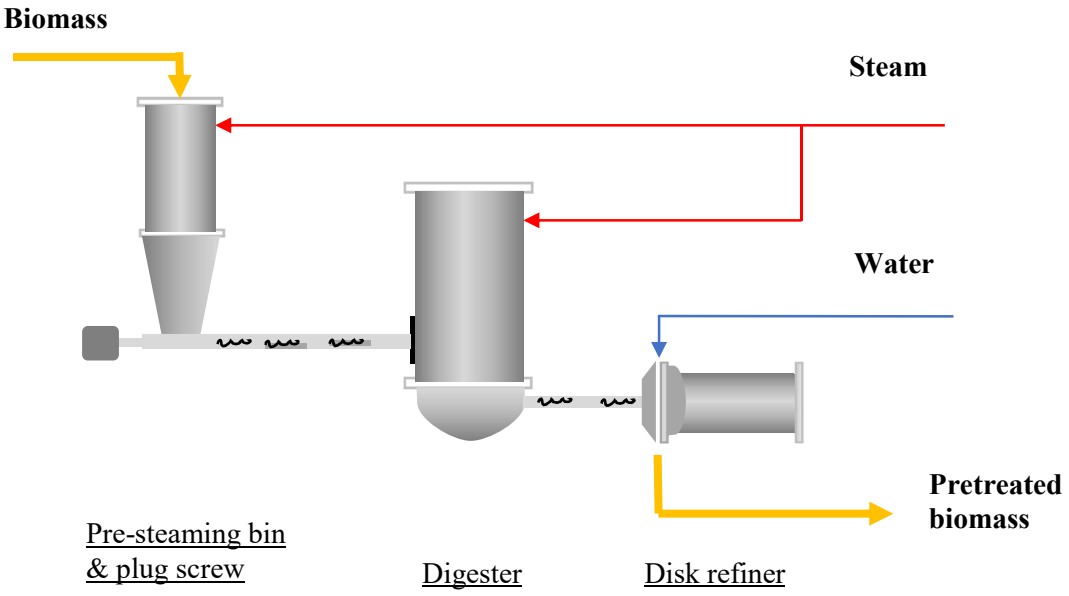

Figure 3.2: Schematic of the pilot plant disk refining system

According to the sub-objectives, two phases of work were applied and shown in Figure 3.3.

Phase 1: Development disk refining pretreatment

In this Phase, the proposed method includes three steps.

(1) Thermal pretreatment with and without chemical addition by the digester

This method is applying a chemical pretreatment before the disk refining pretreatment process. The analysis includes the characterization of chemical composition of the raw material and pretreated biomasses. Moreover, it includes the effect of the pretreated biomass after the thermochemical pretreatment on the energy consumption of disk refining process.

(2) Refining operational parameters: consistency and gap

The selected operational parameters are consistency and gap. The analysis includes energy consumption analysis of disk refining process and fiber length from the pretreated biomass after the thermochemical pretreatment.

(3) Sugar yield evaluation by enzymatic hydrolysis

Sugar yield evaluation by enzymatic hydrolysis from the raw material and pretreated biomasses.

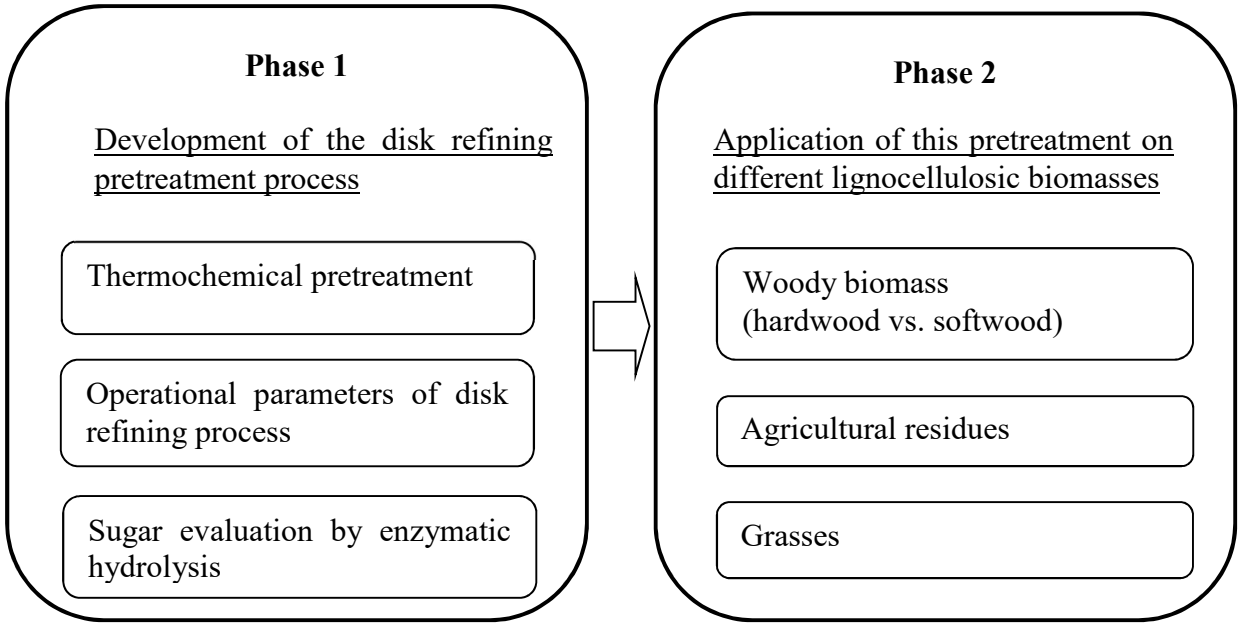

Figure 3.3: Overview of the methodology for the development of disk refining pretreatment

Phase 2: Application of this pretreatment on different lignocellulosic biomasses

According to the results of Phase 1, different lignocellulosic biomass were applied on the process. Four biomasses represent softwood, hardwood, agricultural wastes and grasses. They are black spruce, white birch, alfalfa and corn stover. The analysis factors are as follows:

(1) The effect of the pretreatment process on chemical composition of four biomasses;

(2) The effect of the pretreatment process on energy consumption of four biomasses, as well as to analyze physical properties of fibers;

(3) The effect of the pretreatment process on sugar yield of pretreated biomass.

CHAPTER 4 ARTICLE 1: VALIDATION OF A NOVEL MECHANICAL PRETREATMENT PROCESS FOR THE EXTRACTION OF SUGARS FROM WOODY BIOMASS[#]

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Abstract

This work investigated fermentable sugar production by modifying the traditional mechanical system used by Pulp & Paper Mills as a potential biorefinery step regarding energy consumption and sugar yield. The study explored the effectiveness of thermal pretreatment, with and without the addition of NaOH, followed by a disk refining pretreatment using various gap and consistency operating conditions through a pilot scale disk refining system. The chemical components and sugar streams obtained from woody biomass using thermal and/or chemical refining pretreatments were characterized and analyzed. The energy consumption of the disk refining system was also analyzed. The results show that the effects on chemical components of biomasses are mainly caused by the removal of lignin content in the thermochemical pretreatment with the addition of NaOH (5% w/w dry biomass). The combination of thermochemical and disk refining pretreatments could significantly reduce energy consumption. Moreover, decreasing the refining consistency from 15% to 5% and increasing the refining gap from 0.15 to 1.00 mm further decreased refining energy consumption. At the same time, the thermochemical and disk refining pretreatment significantly increased the sugar yield. This yield; however, decreases as larger gaps are used in the refining process. Therefore, when using existing mechanical refining equipment, a modified thermochemical disk refining pretreatment (TCDRP) can produce a higher sugar yield, by 35%, and lower energy consumption, by 62%, when compared to a typical mechanical refining process.

[#] This article has been submitted to energy & fuels in January 2017.

4.1 Introduction

The bio-based economy aims to produce bioenergy, chemicals, and bioproducts on a large scale [69]. Sugar is a core transitional biofeedstock which is produced using a biorefinery process and can be further converted into biofuels or chemicals. Thus, sugar offers a sustainable alternative to fossil fuel-based chemicals. One of the challenges involved in using lignocellulosic biomass to produce sugar; however, is developing an efficient pretreatment process before the hydrolysis step. This is because of wood biomass's complex composition that primarily includes lignin, hemicellulose, and cellulose.

Traditionally, highly energy-intensive mechanical pretreatment has been considered unsuitable for biofeedstock processing in a biorefinery. However, due to its advantages regarding large production capacity and commercial availability in wood transformation processes, the present work assessed mechanical pretreatment using the existing disk refining (pulping) system as a potential biorefinery step for producing low-cost sugar streams.The mechanical refining system is the core of mechanical pulping processes such as thermomechanical pulping (TMP) and refiner mechanical pulping (RMP). By means of disk refining, the pulp has a high yield of production exceeding 90% [18]. Globally, Canada produces the most mechanical wood pulp, primary because of its dominant mechanical paper grade for newsprint manufacturing. However, since 2000, the North American market for newsprint has declined by 65% since 2000 [70]. Thus, over the ten years preceding 2016, the mechanical pulping industry has faced decling revenue. This means that research on diversified products from the mechanical refining process will significantly affect the future of newsprint mills. Mechanical refining technology has attracted increasing attention as a pretreatment method to improve the enzymatic digestibility of lignocellulosic biomass by overcoming biomass recalcitrance [12]. The application of the newsprint disk refining system will offer great possibilities for converting newsprint mills into biorefinery mills. In addition, the biorefinery industry will benefit from its high productivity, the use of existing infrastructure and an experienced workforce that is available in the newsprint mills. However, in most cases, the mechanical refiner only refers to the post-refining or stock preparation in chemical pulping mills [44]. In 1990, Schell and Harwood [71] showed the mechanical treatment increase sugar yield by reducing the biomass particle size. This treatment; however, was also energy intensive. Also, without removing lignin, mechanically pretreated biomass has a low sugar yield compared to chemically pretreated biomass. Therefore, finding a way to create suitable quality refined biomass

while avoiding high energy consumption is essential for using the newsprint mill disk refining system.

In general, the quality of refined biomass and energy consumption both influence pulp quality. Both of these factors are affected by the refiner disk design, the feed properties and the operational conditions [58].

When using an existing mechanical pulping process, the properties of the feedstocks can be altered for the mechanical process. In fact, the energy consumption needed for pulp production can be reduced using a thermal process involving steam, at about 110-135˚C, and pressure to soften the wood chips [18]. Similarly, in pretreatment, steaming is equivalent to autohydrolysis. In autohydrolysis, an empirical model of severity factor (S) reflects an indication of pretreatment. It is a function of residence time (t, min) and temperature (T, ˚C) [72].

$$S = Log\ t * \exp\frac{T - 100}{14.75}$$

High temperature from 160 to 220 ˚C is very typical for steaming pretreatment [73], while, according to Wang [74], low temperature (less than 140 ˚C) has been proven to open the wood's structure in a mild steam explosion technology. Moreover, adding chemicals during the steaming process is very useful for improving sugar yield by changing the chemical composition of the biomass, as well as reducing energy costs [12]. Alkaline pretreatment, for example, is a delignification and partial hemicellulose removal process (including acetyl groups and uronic acids) [35]. Additionally, the swelling of cellulose can substantially increase the fiber surface area [35]. This pretreatment has a low reaction temperature, no equipment erosion, and few of the environmental issues caused by acid pretreatments, such as sulfite acid pretreatment. Recently, Chen et al. studied the use of alkaline combined disk refining pretreatment for corn stover [13]. To advance biofuel production; however, it is important to evaluate the use of this pretreatment for woody biomass.

Alternatively, modifying the refining process's operational conditions can improve its energy efficiency and the feedstock's sugar yield. The pulp and paper industry has extensively studied methods for reducing energy cost and improving pulp quality. For example, according to the refining theory of defibrillation and defiberation, high consistency refining (HCR) (over 30% w/w pulp) can produce highly defibrillated fibers without reducing fiber length but with a high energy

cost [75]. In contrast, low consistency refining (LCR) (less than 10% w/w pulp) results in more cut fibers but with low energy consumption [76]. Traditionally, because it reduces the fiber length, LCR is not recommended for paper production. When producing sugar via enzymatic hydrolysis; however, the main requirement of refined biomass is to increase the accessibility of enzymes to react with fiber cellulose rather than to separate and maintain single fiber lengths. Also, the distance between the two disks in the disk refiner is refining gap, which significantly affects the fiber length by applying shear force and compression force [23]. Therefore, the study of low consistency and gap of the disk refiner are meaningful for feedstock production.

In this paper, a pilot plant scaled disk refining system for newsprint mills was used to treat hardwood chips for sugar production. The efficiency of mechanical pretreatment using a forward thermochemical pretreatment and modifying the disk refining operation conditions of white birch was studied. The objectives of this work were: (1) to evaluate the effect of thermochemical pretreatment on biomass composition (cellulose, hemicellulose, lignin, and extractives); (2) to assess the effect of thermochemical pretreatment and disk refining pretreatment conditions for reducing energy consumption; (3) to evaluate the yield of sugar production via enzymatic hydrolysis of the biomasses obtained after each pretreatment.

4.2 Materials and methods

4.2.1 Materials

White birch chips from Industries John Lewis (QC, Canada) were used. The chips were further classified by Rader Disc Screen from Rader Companies Inc. (TX, United States) to pass through a 6 mm screen. Then, the chips were washed to remove impurities such as sand and stones. The prepared samples were stored in a cold room until the pretreatment step.

4.2.2 Methods

Fig. 1 shows the overall pretreatment process. It includes the four stages usually used in a pilot scale TMP system from Metso (Finland) (presteaming bin, plug screw, digester, and disk refiner). In this study, the pretreatment process consisted of two-steps: (1) thermochemical pretreatment using the digester; (2) disk refining pretreatment (DRP) using the disk refiner.

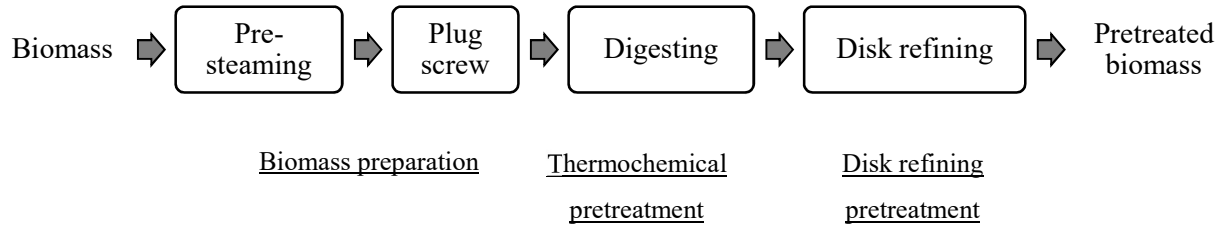

Figure 4.1: Schematic of the pretreatment process

4.2.2.1 Thermochemical pretreatment

Twenty-five kilograms of clean white birch were heated to around 100˚C for five minutes using saturated steam. Next, a plug screw feeder was used to crush the chips and transfer the biomass from the presteaming bin to the digester (0.11 m^3). At the same time, the prepared sodium hydroxide of 5% w/w dry biomass was added to the biomass. The digester was heated using high pressure saturated steam at 140˚C for 30 minutes with the severity of 2.65. The pretreated wood chips were collected from the digester. All of the pretreated samples were characterized for cellulose, hemicellulose, lignin and extractives content via the method of Van Soest, et al. [77].

4.2.2.2 Disk refining pretreatment

After the thermochemical pretreatment, the biomass was collected from the digester. Then, the pilot scale disk refiner (Metso CD-300), equipped with both a fixed and rotating disk, was used for the disk refining pretreatment. In the test, the disk refining pretreatment was carried out under atmospheric conditions with a series of gaps of 0.15 mm, 0.25 mm, 0.50 mm, 0.80 mm and 1.00 mm. The refining pulp consistencies were 5%, 8%, 11%, and 15%. The electrical energy consumption was recorded for the disk refining pretreatment and calculated to net specific energy (NSE) [44]:

$$NSE\ (kW\ \ /ton) = \frac{P_{tot} - P_{no-load}}{M}$$

Where P_{tot}: Total power consumed during refining (kW); $P_{no\text{-}load}$: No load power (kW); M: Dry weight of refined pulp per hour (ton/h).

The pulp collected from the disk refining pretreatment were filtrated using filter papers to remove the wastewater and air dried to about 50% (w/w) humidity. The solid material was stored for characterization, including fiber length by Fiber Quality Analyzer (OpTest Equipment Inc. ON,

Canada) and cellulose, hemicellulose, lignin and extractives content by the method of Van Soest and al. [77, 78].

4.2.2.3 Enzymatic hydrolysis

Enzymatic digestions of the samples obtained from pretreatment were performed in 250 mL shake flasks at 10% solid loading in citric acid buffer with work volume of 125 mL. The addition of ACCELLERASE® DUET enzyme (from Dupont Industry Biosciences, California, USA) was 0.25 mL/dry g of biomass. The enzymatic hydrolysis conditions were 55˚C, 200 rpm, pH=4.8 and 72 h. Tetracycline and cycloheximide were added at 0.5% v/w of biomass to avoid any contamination from microorganisms. Samples obtained at 72 h of hydrolysis were analyzed for total sugar concentration by the method of Miller [79] using dinitrosalicylic acid with reading on a spectrophotometer. The sugar yield is defined as the mass of released reducing sugars during enzymatic hydrolysis divided by the sugars theoretical released from its total carboxylates of hydrolyzed biomasses.

4.3 Results and discussion

4.3.1 Thermochemical pretreatment

Figure 4.2 shows the results of biomass components of the raw material, the thermal pretreated biomasses with and without the addition of NaOH (5% w/w dry biomass, steaming at 140˚C). The thermal pretreatment is designated as TP, and the thermal pretreatment with NaOH is designed as thermochemical pretreatment (TCP).

It is clearly showed that pretreatment methods affects the chemical transformation in ligncellulosic components. Specifically, the lignin content and hemicellulose content in the TCP solids are lower than that of the raw material, around 10% and 5%, respectively. However, the values of the extractives and cellulose content increased in TCP solids compared to the raw material. In contrast to TP, the addition of NaOH in the TCP caused significant delignification. This can be explained by the fact that, during the alkaline pretreatment, sodium hydroxide may promote the rearrangement of the phonate structure, where the ∂-aryl and ∂-alkly ether bonds can be cleaved [80]. The increased extractives are caused by the formation of pseudo-extractives during the pretreatment [35].

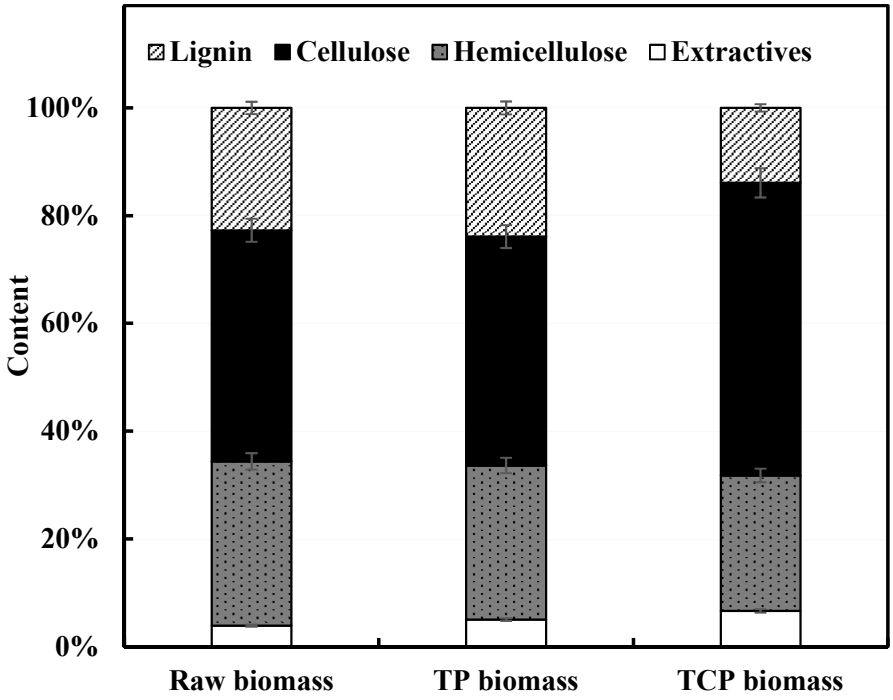

Figure 4.2: Effects of TP and TCP on biomass components

Compared to the traditional alkaline pretreatment using high alkaline loading (10% w/w dry biomass) or the reported method using low temperature deacetylation (<100 ˚C) for 2 hours [13], the thermochemical pretreatment has the following advantages: (1) the lignin extraction by applying a high reaction temperature and a short residence time and (2) the reduction of hemicellulose sugar loss by mild alkaline loading. Typically, 30% or more of hemicellulose is lost by peeling reaction from traditional alkaline pretreatments [13].

4.3.2 Disk refining pretreatment

4.3.2.1 Effect of the thermochemical pretreatment on energy consumption of disk refining pretreatment

Table 4.1 presents the effects of the thermal disk refining pretreatment with and without the addition of NaOH, on the energy consumption of the disk refining pretreatment. The thermal disk refining pretreatment without NaOH is designated by TDRP, and the thermochemical disk refining pretreatment is designated by (with NaOH) is TCDRP.

This table shows that, at given refining conditions, TCDRP consumes less net specific energy than TDRP. Moreover, the smaller the gap, the less energy TCDRP consumes. The energy reduction reaches its maximum at 80%, when the refining gap is at 0.25 mm. This result is competitive with the other reported methods using hot water, dilute acid and SPORL [61].

Adding NaOH during the thermal pretreatment reduces the energy consumption of the disk refining pretreatment. There are two main reasons for this: (1) the dilute alkaline solution causes the wood to swell, thus softens the wood fibers; (2) the partial removal of the lignin by dilute alkaline can produce nanoscopic pores between cellulose microfibril bundles which further weaken the fiber structure [80].

4.3.2.2 Effects of refining consistency and gap on energy consumption of the disk refining pretreatment

Figures 4.3 and 4.4 show the effects of the refining consistency and refining gap of the TCDRP biomass on energy consumption.

Table 4.1: Net specific energy consumption of TDRP and TCDRP

Refining gap (mm)	Net specific energy (kWh/ton)		Energy reduction (%)
	TDRP	TCDRP	
1.00	641	376	40
0.50	1229	682	45
0.25	6363	915	86

According to Figure 4.3, at a given gap size, increasing the refining consistency requires higher specific energy consumption during disk refining than low refining consistency. In the TCDRP, a 15% consistency consumes about 3-5 times more energy than a 5% consistency. Thus, by decreasing the refining consistency from 15% to 10%, the energy consumption decreases around 48-66% depending on the applied gap size. When the consistency is further decreased to 5%, it can further decrease the energy consumption by about 20%. In total, up to 82% of the energy can be saved by adjusting the refining consistency from 15% to 5%.

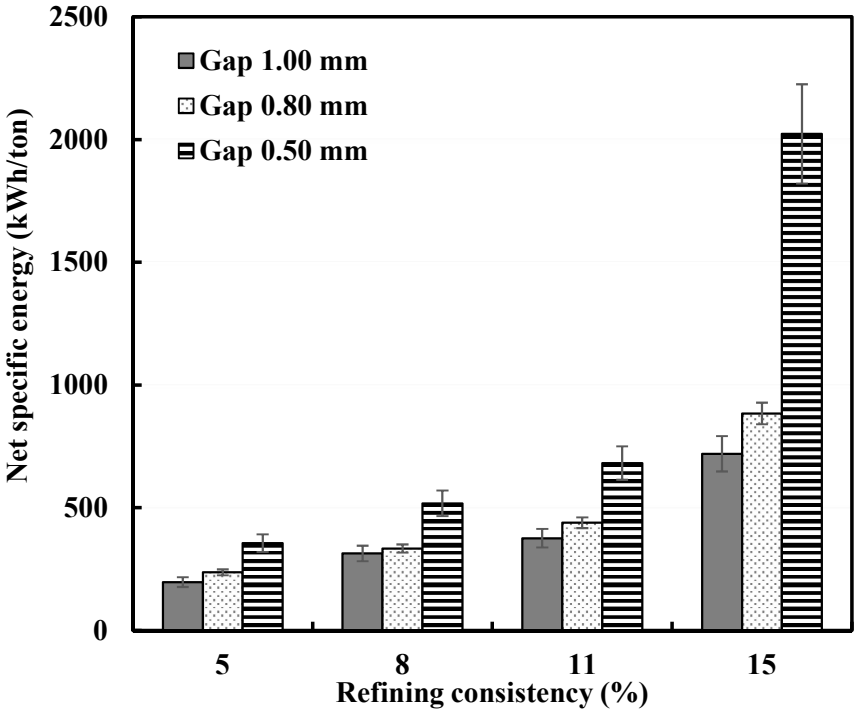

Figure 4.3: The effect of refining consistency on net specific energy

Figure 4.4 shows that for all consistencies, the net specific energy changes as the gap size decreases. Particularly, when the gap is 0.15 mm, about 1-3.5 times more energy is consumed than when the refining gap is 1.00 mm. This can be explained by the fact that the net energy consumption is inversely proportional to the gap from Luukkonen [81]. Thus, increasing refining gap size from 0.15 mm to 1.00 mm reduces the energy consumed by around 70-82%. This corresponds with the reference reported energy cost saved over 80% by adjusting the refining gap and consistency [61].

Overall, both a high refining consistency and a small gap consume a lot of energy. In general, refining at a high consistency and using a small gap usually causes significant friction between the fibers and the disks. It not only develops the fiber properties, also increases the temperature in the refining zone. Thus, the heat loss is caused by steam evaporation which is generated by the high temperature in the refining zone [23].

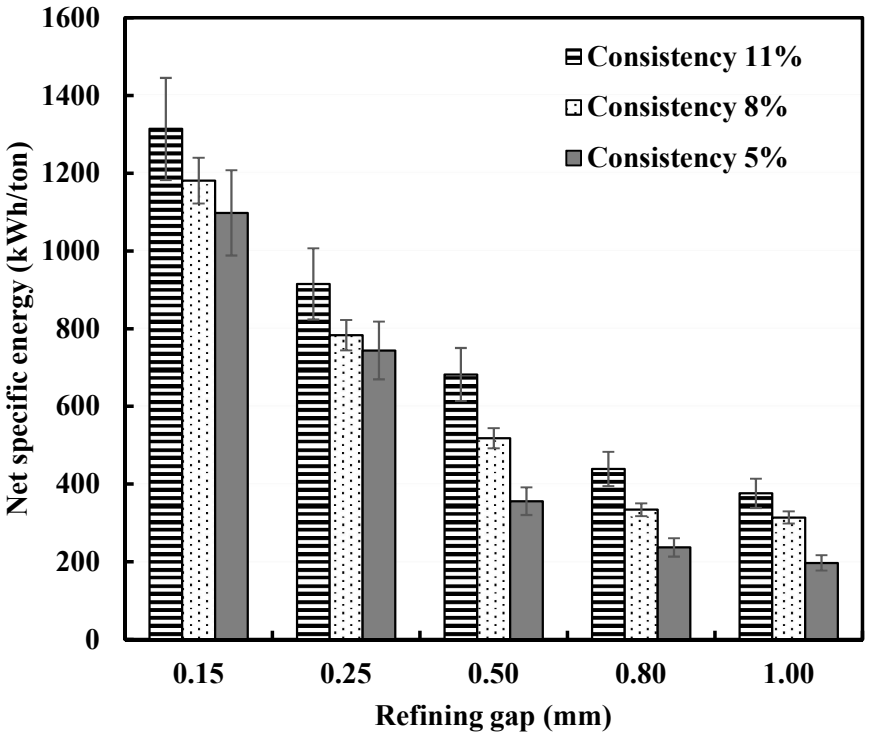

Figure 4.4: The effect of refining gap size on net specific energy

4.3.2.3 Effects of refining consistency and gap size on fiber length

Figure 4.5 presents the effects of the disk refining gap on the fiber length for the different consistencies (5, 8 and 11%) of the pretreated biomass.

Firstly, there is a critical gap size, at approximately 0.25 mm, below which fiber length declines dramatically. Specifically, the fibers are shorter when the gaps are smaller. This is consistent with previous findings [81]. Because the high energy associated with the small gap size can increase the energy consumed on the fibers, this can generally affect fiber size by two pathways [23]: (1) size reduction, i.e. short fibers, by cutting and peeling (the friction between fibers or the fibers and the disks causes the surface layer to peel off); (2) fines, i.e. the fibrils generated during cutting and peeling.

Secondly, the refining consistency did affect fiber length in this study, except for a slightly increase of fiber length at a refining consistency of 5% compared to the other consistencies (8% and 10%). This was possible caused by insufficient refining.

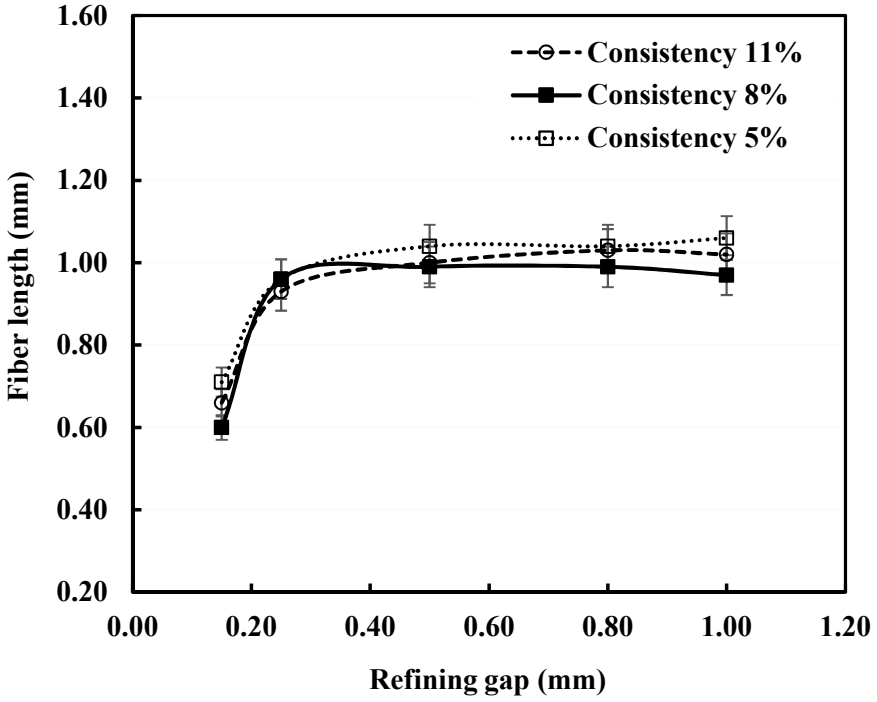

Figure 4.5: Effects of refining consistency and gap on fiber length

4.3.3 Enzymatic hydrolysis

4.3.3.1 Effect of thermochemical pretreatment on sugar yield

Figure 4.6 describes the sugar yield of the pretreated biomasses from raw material, TP (i.e. without the addition of NaOH) and TCP (i.e. with the addition of NaOH 5% w/w dry biomass).

The sugar yield of the raw biomass was 4.45%, and it can be increased to 9.79% and 34.35% by TP and TCP respectively. Particularly, compared to the TP biomass, the TCP biomass had a rapidly increase in sugar yield. This may be because NaOH can partially degrade lignin, which inhibits enzymes by blocking the reactions of enzymes with carbohydrates [82]. Therefore, thermochemical pretreatment improves the enzymes' ability to access the cellulose.

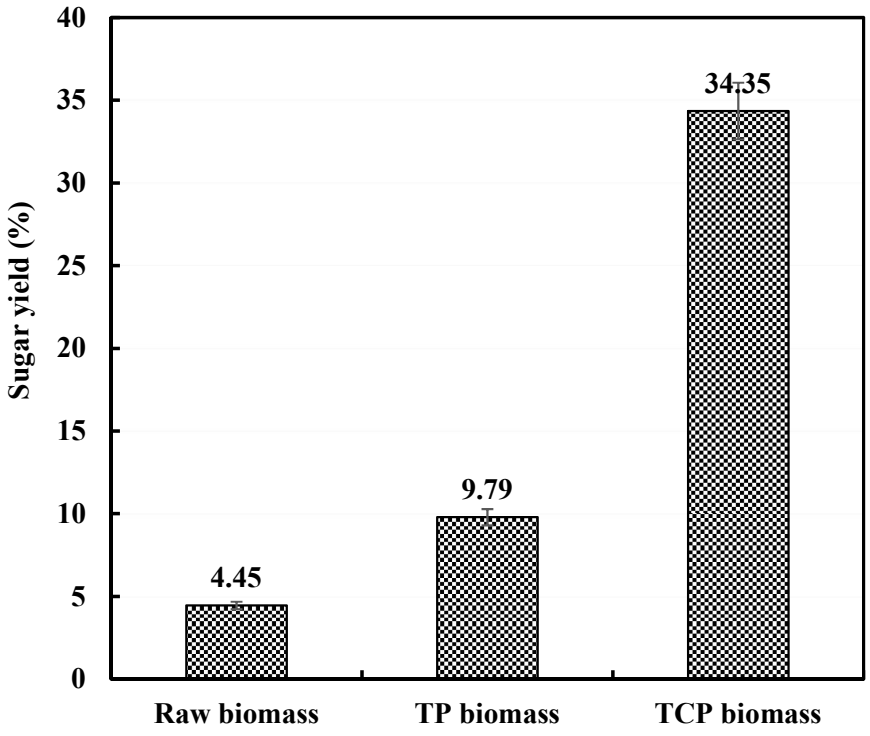

Figure 4.6: Effects of TP and TCP on sugar yield of pretreated biomass

4.3.3.2 Effects of fiber length and gap on sugar yield

Figure 4.7 shows the sugar yield from the TCDRP biomass using various refining gaps.

Firstly, the refining pretreatment in the TCDRP improved the sugar yield about 6-13% depending on the refining gap, compared to the TCP biomass. This confirms that the disk refining pretreatment can enhance the enzymatic hydrolysis yield [11],[24]. This is because refining significantly accelerates the hydrolysis reaction rates by increasing the interactions between cellulose and enzymes.

Secondly, the smaller gap size the TCDRP used, the higher the TCDRP sugar yield. For example, when the gap size was reduces from 1.00 mm to 0.15 mm, the sugar yield increased from 39.06% to 47.22%. However, there is still an increase in sugar yield when the fiber did not decrease, when the refining gap was larger than 0.25 mm. This means that sugar yield was not directly proportional with the fiber length. A reduced fiber length can increase the available fiber surface area which is the most relevant factor to increasing enzyme accessibility [50]. Thus, the increased sugar yield

may firstly be caused by the increased available fiber surface area caused by the separation of fiber bundles, then further increased by numbers of short fibers and newly generated fiber surfaces.

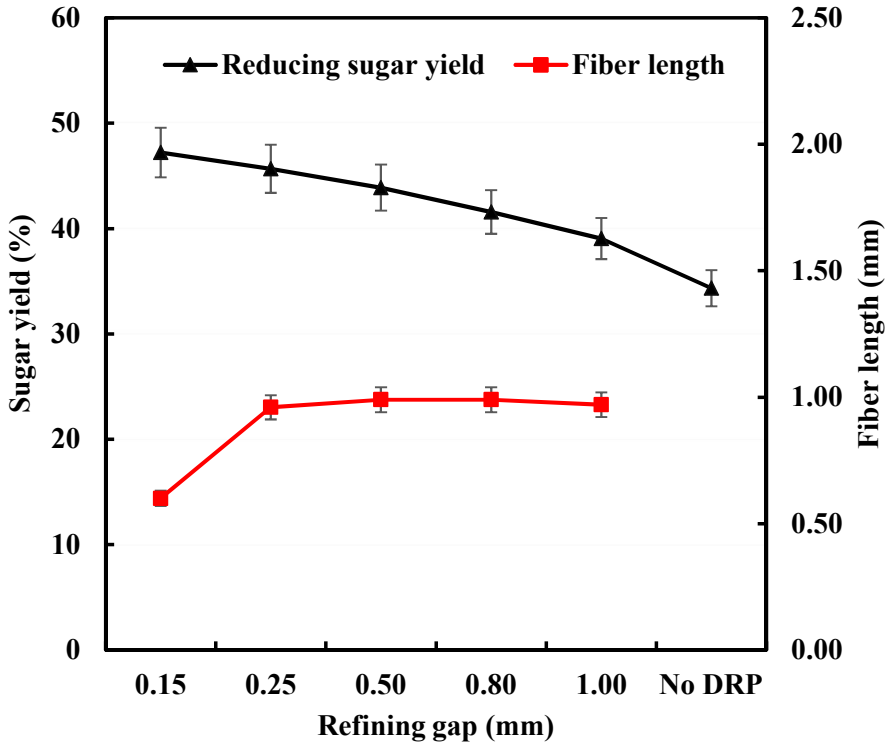

Figure 4.7: The effect of refining gap on the sugar yield and fiber length of the TCDRP biomass (Refining consistency 8%)

4.3.3.3 Effects of refining consistency and refining energy on sugar yield

The sugar yield of the TCDRP biomass with various refining consistencies is shown in Figure 4.8. At a given refining gap, lowering the refining consistency did not affect the sugar yield, except when the consistency was less than 8%. The result is consistent with the trend of fiber length with a low refining consistency.

Figure 4.9 shows the relationship between energy consumption and sugar yield. On one hand, decreasing the refining energy resulted in a slowly reduction of sugar yield in a range of 7-11%. This was mainly caused by the gap size. Thus, a larger refining gap is suggested for intensive energy saving. On the other hand, the refining consistency of 5% had a slightly lower sugar yield than other refining consistencies. Therefore, from an economical point of view, an 8% refining consistency is suggested to avoid the decrease in sugar yield and to maintain low energy consumption.

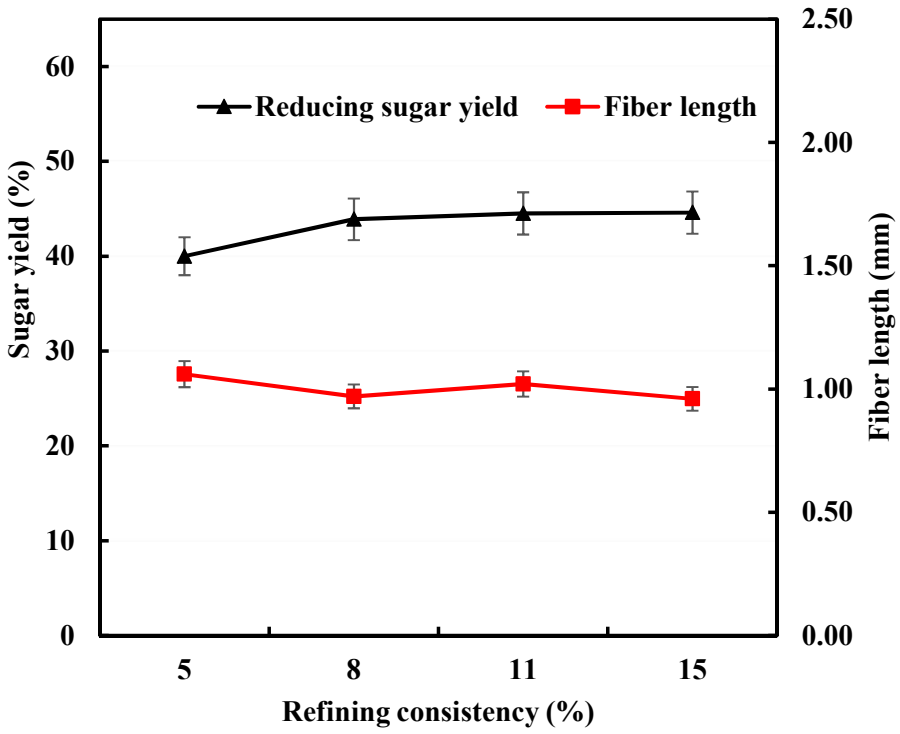

Figure 4.8: The effect of refining consistency on the sugar yield and fiber length of the TCDRP biomass (Refining gap 0.50 mm)

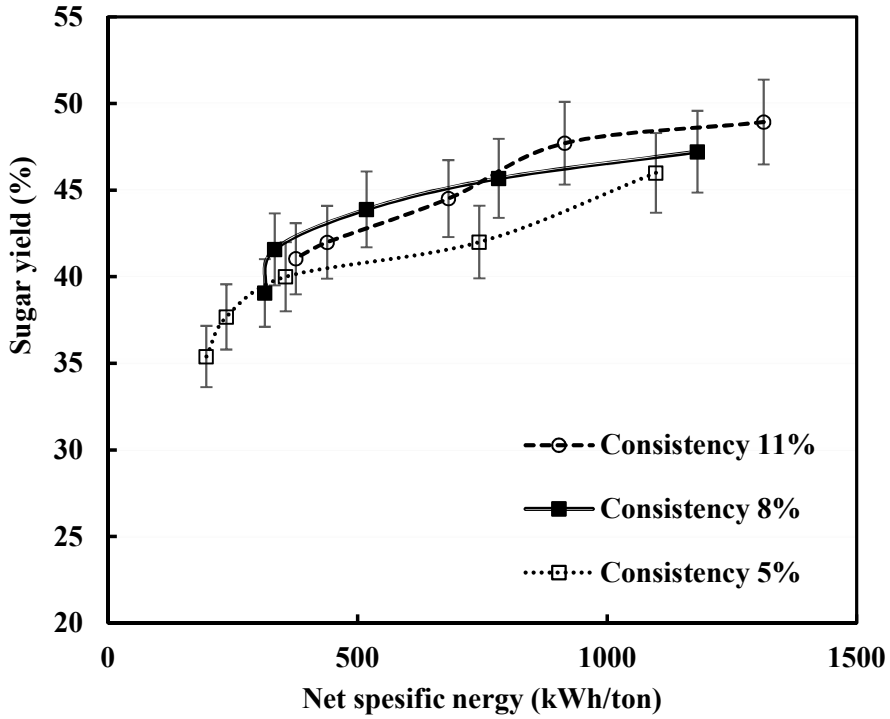

Figure 4.9: The relashionshiop between net spricific energy and sugar yield of TCDRP

4.3.4 Comparison between the TCDRP and the typical TMP process

Table 4.2 shows that the comparison is between the modified pretreatment TCDRP and a conventional TMP process (without modification) for white birch chips in terms of energy consumption and sugar yield. It also includes the results of the raw material.

Compared to the raw material for sugar extraction, the TMP pretreatment process consumed 872 kWh/ton, but only slightly increased the sugar yield. This may be caused by the short steaming time and high refining consistency. By modifying the typical TMP process, the TCDRP process used a two-step pretreatment: (1) thermochemical pretreatment with a longer steaming time (30 minutes) and the addition of NaOH (5% w/w dry biomass), (2) operation conditions including a low refining consistency (8%) and a larger refining gap (0.80 mm). Table 4.2 shows that the TCDRP significantly reduced the energy consumption by 62% and achieved a higher sugar yield, around 35%, than the TMP. These results further prove that through the modification of an existing mechanical refining system, mechanical pretreatment can obtain a high sugar yield with a low energy cost.

Table 4.2: On energy consumption and sugar yield of different pretreated process

White birch	Sugar yield (%)	Net specific energy (kWh/ton)
Raw biomass	4.45	N/A
TMP[a]	5.70	872
TCDRP[b]	41.15	334

a: TMP: thermomechanical process (140˚C, 5 minutes, refining gap 1.00 mm, refining consistency 23.4%);
b: TCDRP: thermochemical disk refining pretreatment process (140 ˚C, 30 minutes, refining gap 0.80 mm, refining consistency 8 %)

4.4 Conclusions

Using the existing mechanical refining technology, a new pretreatment process with a low energy consumption and a high sugar yield was developed by combining a thermochemical pretreatment and a refining pretreatment (TCDRP). By adding 5% of NaOH (w/w dry biomass) to TCDRP at 140˚C, the degraded partial lignin resulted in an increase of cellulose content in the substrates. Adding NaOH to the TCDRP pretreatment reduced the energy consumption. Moreover, reducing

the refining consistency from 15% to 5% and increasing the refining gap from 0.15 mm to 1.00 mm can further reduce the energy cost. The refining gap has the greatest impact on fiber length, in comparison with refining consistency. Sugar yield can be increased by adding NaOH to the thermal pretreatment. However, the larger refining gap has a negative effect on sugar yield; the reduced refining consistency slightly reduces sugar yield. Therefore, the TCDRP is a two-step pretreatment process. The first step is the thermochemical pretreatment at 140 ˚C for 30 minutes with an addition of NaOH (5% w/w dry biomass). The second step is the disk refining pretreatment under the operational conditions with a refining consistency of 8% and a large refining gap of 0.80 mm. Compared to the typical TMP process, this process not only improves the sugar yield by 35% but also reduces the specific energy consumption by about 62%.

4.5 Acknowledgement

Authors are grateful for Mr. Alain Marchand and Bryan Brousseau for their excellent assistance. It is also greatly thankful for the grant support from BiofuelNet.

4.6 Abbreviations

TMP Thermomechanical pulping; DRP Disk refining pretreatment; TP Thermal pretreatment; TCP Thermochemical pulping; TCDRP Thermochemical disk refining pretreatment; NSE Net specific energy.

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CHAPTER 5 EFFECT OF MECHANICAL PRETREATMENT FOR ENZYMATIC HYDROLYSIS OF WOODY RESIDUES, CORN STOVER AND ALFALFA

5.1 Introduction

Lignocellulosic biomass is the most abundant, sustainable, renewable and environmentally friend resource that can be used to minimize the carbon cycle impact. Compared to starchy biomass or sucrose, the non-food lignocellulosic biomass has been recognized as the most potential resource in biorefineries [1, 3, 83-85].

Woody residues are lignocellulosic biomasses, such as sawdust and wood branches. It is commonly used in industrial applications, such as pulp and paper manufacturing. According to a subdivision of the division spermatophytes (plants with seeds), wood are known as softwood such as spruce, pine, and fir, and hardwood such as birch, aspen and maple [18]. Typically, softwood fibers are about 3 millimeters in length and about 20 to 35 micrometers in width [86]. By contrast, fibers from hardwood are generally about 1 mm in length and not as thick as softwood [86].

Other sources of lignocellulosic biomass are grass and agricultural residues, for example, alfalfa and corn stover. Alfalfa (medicago sativa) is a perennial plant, the third most important field crop after corn and soybeans [87]. It is usually cultivated as an important forage crop [88]. Recently, utilization of the byproduct alfalfa stems for industrial use has just started to attract researchers' attention. It has been considered as a potential feedstock for production ethanol and other chemicals through biological and combustion conversion due to its high biomass yield, high-value byproducts and improvement of soil nutritious [87, 88].

Corn stover is the residues after corn harvest, such as stalks, leaves, and cobs that remain in fields after the corn harvest. In America, corn stover has about 120 millions tons annually, as the largest quantity of biomass residue [89]. In Québec (Canada), corn grain is cultivated on about 4000 km^2/year since 2007 (Institut de la statistique du Québec, 2011). Corn stover represents a yearly biomass potential of 3 million tons (considering an estimated yield of more than 800 t dry matter/ km^2) [89].

Nowadays, an efficient pretreatment process before the hydrolysis step is still one challenge for sugar production from lignocellulosic biomass. Because lignocellulosic biomass has more of the complex structure and chemical components than the starchy based biomass to resist enzymatic deconstruction [24]. In these years, many pretreatment methods have been developed and can be classified as chemical, physical, biological and other pretreatment. Mechanical pretreatment is one of the physical pretreatment, which is able to increase sugar yield by particle size reduction. Traditionally, it is considered not suitable for sugar production because of its energy intensive and low sugar yield by comparison with other methods. However, according to its high productivity, commercial availability, and low environmental impacts, mechanical pretreatment is getting more attentions through process modification and optimization.

Disk refining technology has attracted increasing attention as a pretreatment method for lignocellulosic biomass to improve enzymatic digestibility by overcoming biomass recalcitrances [12]. Disk refining system is the core of mechanical pulping processes such as TMP (thermomechanical pulp) and RMP (refiner mechanical pulp) [18, 23]. Canada's mechanical pulping industry, mainly for newsprint production, has faced a declined market of newsprints by 65% in North American demand since 2000 [70]. In fact, 30 of newsprint mills have shut down from 2008 to 2013 in Eastern Canada and Northeastern America [90]. Therefore, many potentially idled mechanical equipment are available for pretreatment in biorefineries.

In our previous research, a thermochemical disk refining pretreatment (TCDRP) has been developed to enhance enzymatic hydrolysis efficiency of white birch. In this chapter, four lignocellulosic biomass species including corn stover, alfalfa, white birch and black spruce are studied. The objectives of this works are shown as follows:

(1) to compare the effect of the pretreatment process on chemical composition of four biomasses;

(2) to examine the effect of the pretreatment process on energy consumption of four biomasses, as well as to analyze physical properties of fibers;

(3) to evaluate the effect of the pretreatment process on sugar yield of pretreated biomass.

5.2 Materials and methods

5.2.1 Materials

White birch chips from Industries John Lewis (QC, Canada) and black spruce from Trois-Rivières Kruger mill (QC, Canada) were utilized. The chips were further classified by Rader Disc Screen from Rader Companies Inc. (TX, United States) to pass through the screen of 6 mm. Then washing process was applied to remove the impurities such as sand and stone. And finally the prepared samples were stored in a cold room until the pretreatment step.

Corn stover provided by Ferme Olivier and Sebastien Lépine of Agrosphere Company (QC, Canada) and alfalfa from TH-Alfalfa Inc. (QC, Canada) were used. The received corn stover and alfalfa were stored and directly used in pretreatment process.

5.2.2 Methods

Figure 5.1 shows the overall TCDRP processes. For woody biomasses, it includes four steps usually utilized in a pilot scale TMP system (presteaming, plug screw, digesting, disk refining) from Metso (Helsinki, Finland). For agricultural wastes and grasses, a mixter was used before digester.

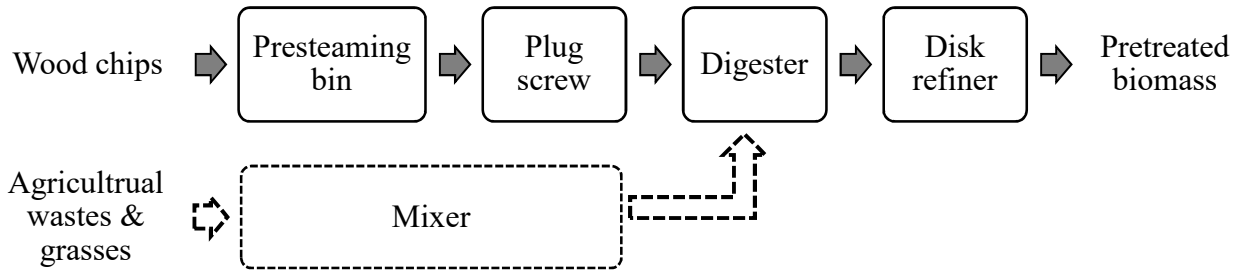

Figure 5.1: Schematic of the pretreatment process for lignocellulosic biomass

5.2.2.1 Thermochemical pretreatment

(1) Black spruce and white birch

In the case of woody biomass (Figure 5.2), the fresh chips about 25 kg were heated at around 100˚C for 5 minutes by saturated steam. Then plug screw was followed to remove some water, crush the chips and transfer the biomass from presteaming bin to the digester (0.11 m^3). At the same time, the prepared hydroxide sodium of 5% w/w dry biomass was transferred to biomass by the same flow rate of biomass. Then the digester was heated by high pressure saturated steam for 30 minutes

with various steaming temperature: 140˚C with the severity of 2.65. The biomass was collected from the digester after pretreatment.

(2) Alfalfa and corn stover

In the case of agriculture wastes and grasses (Figure 5.2), the soda of 5% (w/w dry biomass) was prepared and mixed with biomass by a mixer. Around 10 kg (dry mass) mixed biomass was loaded in the 0.11 m^3 digester. Then it was heated by a high-pressure saturated steam for 30 minutes with various steaming temperature: 140˚C with the severity of 2.65. The biomass was collected from the digester after pretreatment.

All pretreated samples (Figure 5.3) were subjected to characterize the content of cellulose, hemicelluloses, lignin and extractives by the method of Van Soest Van Soest, et al. [77] and al. (1991).

Figure 5.2: White birch chips (a), black spruce chips (b), alfalfa (c), and corn stover (d)

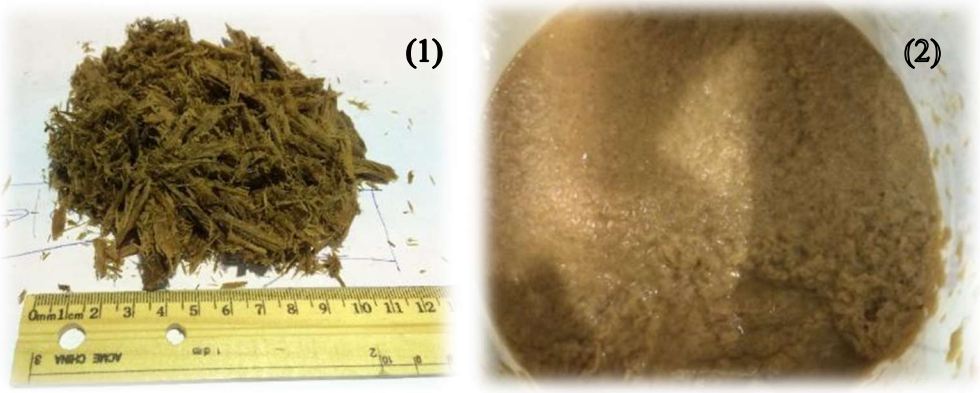

Figure 5.3: Samples of TCP biomass (1) and TCDRP biomass (2)

5.2.2.2 Disk refining pretreatment

Pilot scale Metso CD-300 disk refiner equipped with a fixed disk and a rotating disk was used in disk refining pretreatment. The clearance between the rotating and stationary disks is between 0 mm to 3 mm. The consistency of refining can be adjusted by flow rate of water. The pretreated wood chips were collected from the (0.11 m^3) digester. In the test, the disk refining pretreatment was carried out under atmospheric conditions with a series of gap 0.15 mm, 0.25 mm, 0.50 mm, 0.80 mm and 1.00 mm and the pulp consistency was 8%. The electrical energy consumption for disk refining pretreatment was recorded and calculated by the net specific energy (NSE) [44]:

$$NSE\ (kWh/ton) = \frac{P_{net}}{M} = \frac{P - P_{no\ load}}{M}$$

Where P: Refining Power (kW); $P_{no\text{-}load}$: No load power (kW); M: Dry weight of refined pulp per hour (ton/h).

The pulp from disk refining (Figure 5.3) was collected as well as filtrated by the filter paper to remove the wastewater and air dried to about 50% humidity. The solid material from filtration was stored for characterization and enzymatic hydrolysis.

All samples obtained after refining pretreatment were subjected to characterize shives content by Masterscreen (Pulmac Systems International Inc., QC, Canada), fiber length by Fiber Quality Analyzer (OpTest Equipment Inc. ON, Canada) (Figure 5.4), and chemical composition contents by the method of Van Soest and al. [77].

Figure 5.4: Fiber quality analyzer (1) and Masterscreen (2)

5.2.2.3 Enzymatic hydrolysis

Enzymatic digestions of the substrates from pretreatment were performed in 250 mL shake flasks at 10% solid loading in citric acid buffer with work volume of 125 mL. ACCELLERASE® DUET enzyme (from Dupont Industry Biosciences, California, USA) was added as 0.25 mL/dry g of biomass. It has endoglucanase (2400-3000 CMC U/g), beta Glucosidase (> 400 pNPG U/g), and Xylanases (>3600 ABX U/g). The enzymatic hydrolysis conditions were 55 ˚C, 200 rpm, pH=4.8 and 72 h. The tetracycline and cycloheximide were added as 0.5% v/w of biomass to avoid all contamination of microorganisms. Samples obtained at 72 h were analyzed for total sugar concentration by the method of Miller [79] using acid dinitrosalicylic with reading on a spectrophotometer. The reducing sugar yield is defined as the percentage of released sugars during enzymatic hydrolysis after 72 h in the hydrolyzed biomass based on the theoretical sugars released by its total carboxylates.

5.3 Results and discussion

5.3.1 Chemical composition

5.3.1.1 Effect of TCDRP pretreatment on extractives content of different biomass

Figure 5.5 shows the extractives content in the original biomass and the pretreated biomass by thermochemical pretreatment (TCP) and thermochemical disk refining pretreatment (TCDRP).

In the raw alfalfa, the component of the extractives accounts for 37.4% in total mass. This value is 17.8% for the raw corn stover, 5.8% for the raw black spruce and 3.8% for the raw white birch. By the TCP, the extractives content increases slightly for all biomasses. However, it drops marginally by the TCDRP.

Firstly, the amount of extractives in plants is significantly affected by species of biomass. Black spruce and white birch are two species of woody biomass, which usually has low extractives content than that in corn stover and alfalfa. Because extractives are a variety of chemical components in lignocellulosic biomass [91], such as inorganic materials, carbohydrates, phenols, aromatics, hydrocarbon, lipids, fats, and waxes. Black spruce, as one species of softwood, has more resin acids and monoterpenes than white birch. But corn stover and alfalfa have more protein and starches.

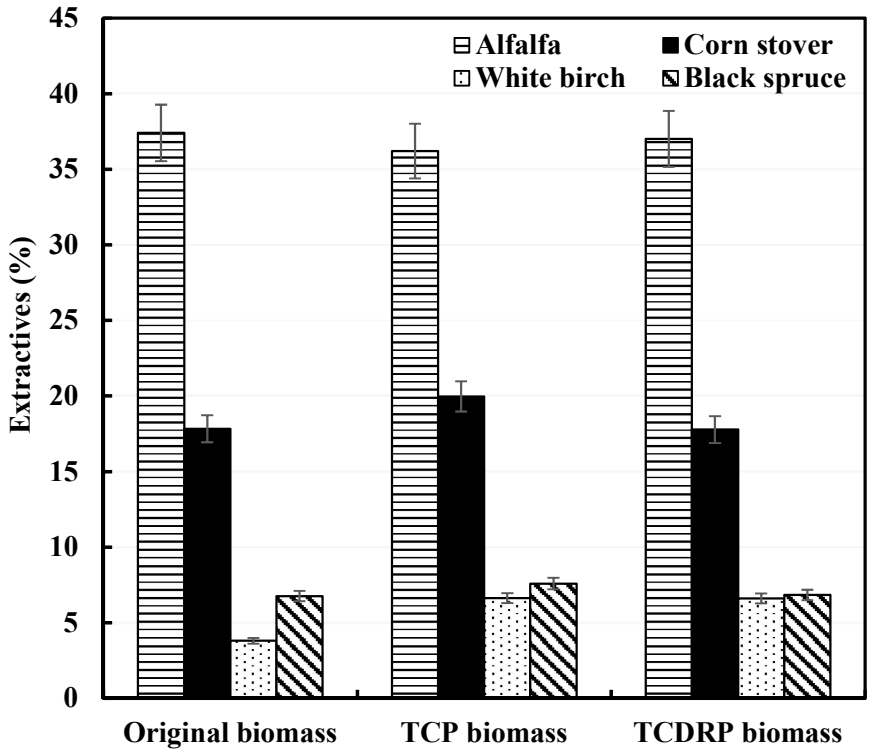

Figure 5.5: Extractives contents of raw biomasses and pretreated biomasses

Then to all biomasses, the chemical pretreatment results in a higher value of extractives than without pretreatment, i.e. original biomass. But, the TCDRP shows the content of extractives could be removed slightly. Because extractives are not chemical that can bound to components of biomass. Thus they can be extracted by various solvents such as water, ethanol, benzene, toluene and their

mixtures [91]. In addition, during the pretreatment, there is a possibility to form of pseudo-extractives which are organic structures usually quantified together with native extractives from biomasses. However, the additional refining pretreatment after the TCP acts like a washing process by low consistency refining. As a result, it slightly removes some dissolved extractives.

5.3.1.2 Effect of pretreatment process on hemicelluloses of different biomasses

Figure 5.6 shows hemicelluloses contained in original biomasses, TCP biomasses, and TCDRP biomasses. Hemicelluloses contents in raw materials are shown as follows: 33.9% in corn stover, 29.7% in white birch, 17.6% in black spruce and 16.3% in alfalfa. The TCP biomasses have less hemicellulose content than the original samples. For instance, the declined percentage of hemicelluloses content is about 4% for white birch, 2% for alfalfa and 2% for black spruce. However, the highest amount (33.9%) of hemicelluloses contained in corn stover is degraded around 12%. At last, compared to the TCP biomasses, the TCDRP biomasses don't play much effect on hemicelluloses content for all biomasses.

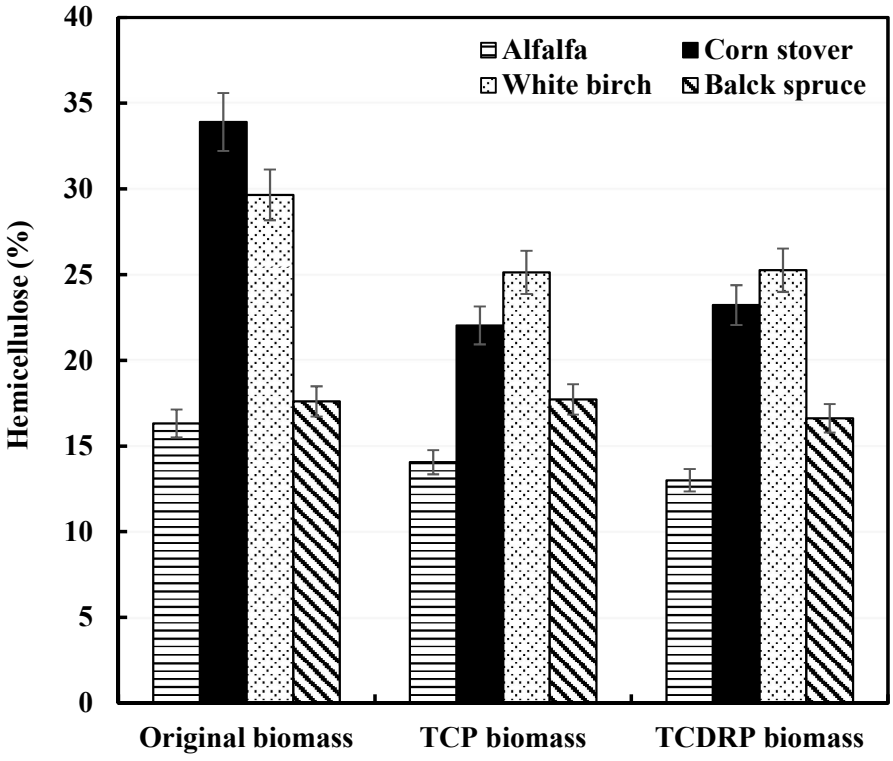

Figure 5.6: Hemicellulose contents of raw biomasses and pretreated biomasses

Hemicelluloses highly branched structure, and the presence of acetyl groups connected to the polymer chain, as well as the lack of crystalline structure [21]. Moreover, hemicelluloses are a

family of polysaccharides such as arabinoxylans, glucomannans, galactans, and others [92]. During the TCP, the acetyl groups connected to the polymer chain are easy to remove and the degradation of hemicelluloses can be caused by the peeling reaction at a low temperature. The amount of degraded hemicellulose are caused by the various composition and structure depending on their sources and the extraction methods [92].

5.3.1.3 Effect of pretreatment process on lignin of different biomasses

The lignin contents from original biomasses, TCP biomasses, TCDRP biomasses are shown in Figure 5.7.

According to the results for initial biomasses, we could see that the highest lignin content is from black spruce (25.4%), then followed by white birch (23.9%), alfalfa (13.7%) and corn stover (7.4%). The TCP biomasses have lower lignin contents than original biomasses. Especially for woody biomass, black spruce and white birch lose lignin content by 8.0% and 10.0% respectively. In comparison with the thermal-mechanical process, additional disk refining treatment in TCDRP almost maintains the amount of lignin in total mass. Thus, the final lignin content in four biomasses are 5.9% (corn stover), 11.6% (alfalfa), 12.8% (white birch), and 17.7% (black spruce).

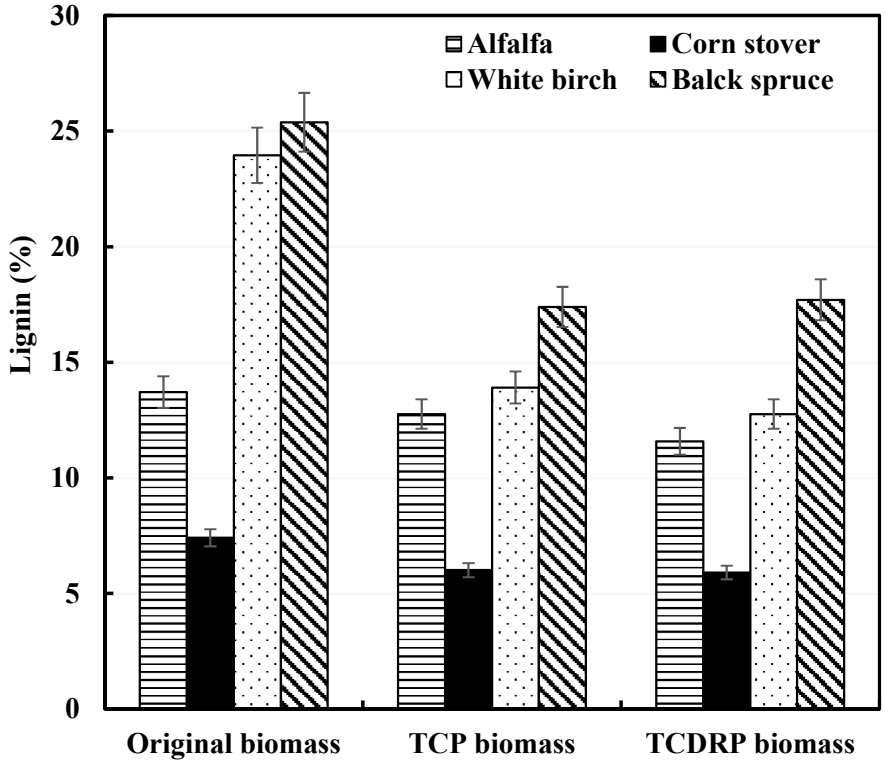

Figure 5.7: Lignin contents of raw biomasses and pretreated biomasses

Like the other components, lignin is also different in various lignocellulosic species. Generally, as the most complex natural polymer, lignin is an amorphous three-dimensional polymer with phenylpropane units as the predominant building blocks. More specifically, the mainly types of structures are p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [21]. Due to the reaction between NaOH and lignin that further breaks macromolecules lignin into small macromolecules, there is a loss of lignin content in TCP biomasses. The degradation of lignin is less in softwood with up to 90% coniferyl alcohol than that in hardwood made up of varying ratios of coniferyl and sinapyl alcohol type of units [21]. Overall, lignin content in corn stover is the lowest because of its low amount of lignin in raw material, although a significant amount of lignin is degraded by NaOH for woody biomass.

5.3.1.4 Effect of pretreatment process on cellulose of different biomasses

Figure 5.8 shows cellulose contents in original biomasses, TCP biomasses, TCDRP biomasses. Firstly, the original black spruce has more cellulose than the original white birch. The cellulose in corn stover is higher than that in alfalfa. Then the cellulose component in all biomasses after both TCP and TCDRP are increased, compared to that in raw materials.

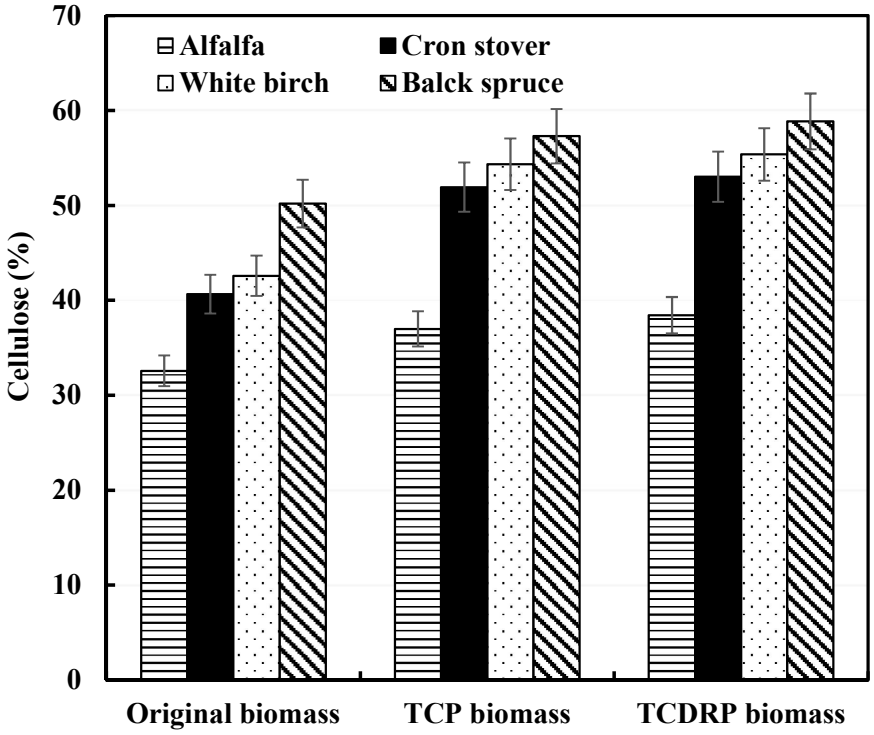

Figure 5.8: Cellulose contents of raw biomasses and pretreated biomasses

As we know, cellulose is constituted by the *β-1,4*-polyacetal of cellobiose (*4-O-β-D-glucopyranosyl-D-glucose*). Cellulose is a polymer which constituted by the unit of glucose. It is found in both the crystalline and the non-crystalline structure [21]. It's not degraded by its compacted crystalline structure except extensive swelling and dissolution of the low molecular weight fraction of the polymer (DP < 200) in alkaline conditions. However, the reduction of other components in biomass such as lignin is the main reason for rising cellulose content by pretreatment.

5.3.2 Energy consumption

5.3.2.1 Effect of pretreatment process on energy consumption of different biomasses

Figure 5.9 shows net specific energy at a series of refining conditions for the biomass that were pretreated by the TCDRP. Here we only consider the electrical power in refining step.

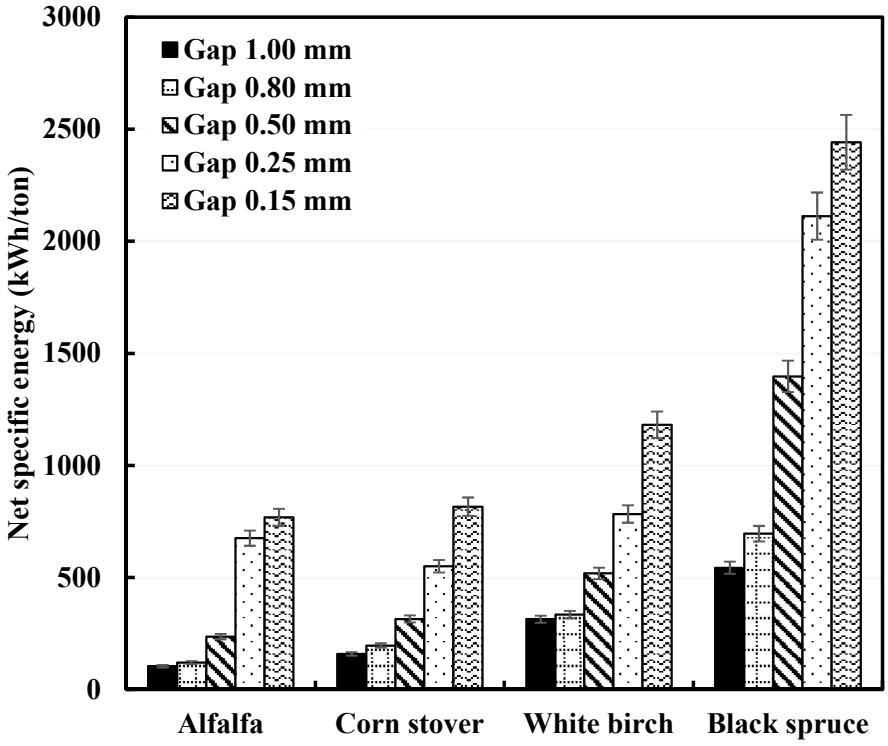

Figure 5.9: Relationships between net specific energy and various lignocellulosic biomasses

Note: the energy consumption does not include the thermal energy, only electricity power for refining process

On the one hand, energy consumption significantly increases with a smaller gap from 1.00 mm to 0.15 mm. This is well in accordance with previous findings [81]. That is, the power is

proportional with 1/G. When the gap reduces, more energy is applied to fibers, i.e. high refining intensity. In physical pretreatment, wood chips or agricultural pieces are separated and destroyed to small pieces.

On the other hand, at the same refining condition, black spruce consumes more energy than the other biomasses. For example, at the gap of 0.25 mm, the specific energy of black spruce is 2442 kWh/ton, which is three times higher than that of alfalfa (768 kWh/ton).

Therefore, the energy consumed of the TCDRP for different biomasses shows that refining energy is not only affected by refining gap, but also affected by biomass species. The result is consistent with literatures [58, 93].

5.3.2.1 Effect of energy consumption on fiber length and shives content of different biomasses

To further understand the refining energy consumption of the TCDRP for various biomasses, it is important to study physical properties of fibers.

- Shives content

Figure 5.10 shows shives contents in corn stover and alfalfa with various refining energy. It shows shives contents are from high to low with higher refining energy. Especially, the shives reduction is around 39% for alfalfa while it is approximate 12% for corn stover.

Shives are the fiber bundles that are not completely separated by the refining pretreatment. Thus, with more refining energy by smaller gaps, especially for 0.25 mm and 0.15 mm, the bundles can be shortened or separated into small fibers or single ones.

- Fiber length

To further compare between agricultural biomass and woody biomass, Figure 5.11 describes fiber length (from shives removed pulps) at various refining energy. At first, it shows with increasing refining energy, the fiber length of all biomasses has no significant changes, but it has a rapid decline (once the gap behinds critical gap size). The relationship between energy and fiber length was studied by Luukkonen et al. [81]. It confirms that refining has two effects on fibers: the separation from big biomass pieces to fiber bundles; fiber size reduction.

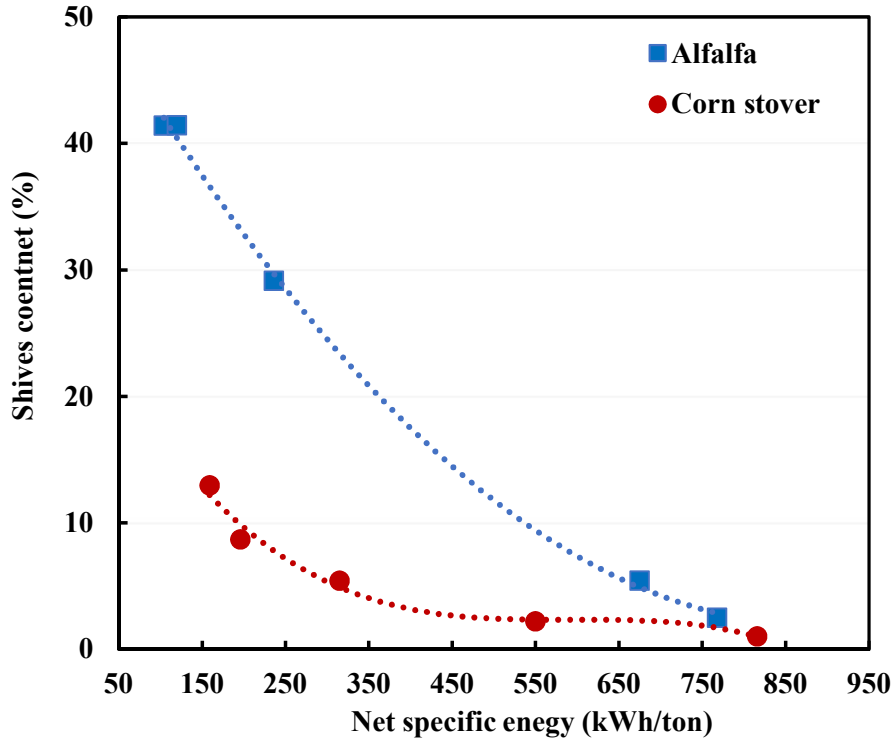

Figure 5.10: Relationship between shives content (w/w) and net specific energy

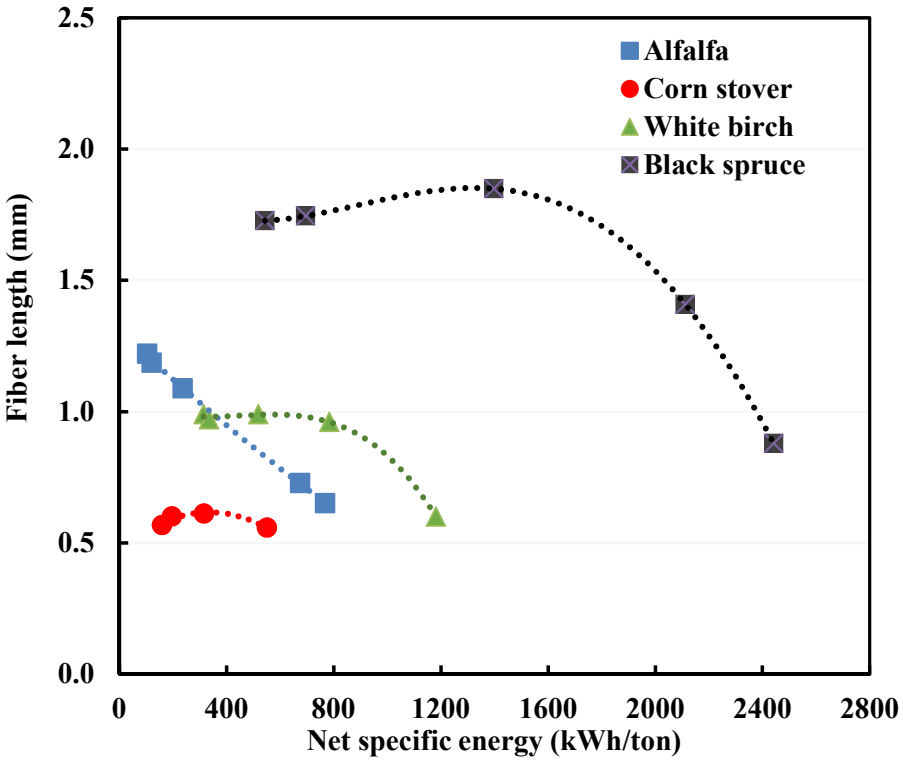

Figure 5.11: Relationships between fiber length and various lignocellulosic biomasses

Secondly, when the energy consumption is same, black spruce achieves the highest fiber length while corn stover has the shortest fiber length. It shows the biomass characters affects the energy consumption.

5.3.3 Sugar yield by enzymatic hydrolysis

- Thermochemical pretreatment

To study the effect of this TCP process on different biomasses in sugar yield, Table 5.1 lists the sugar yield of raw materials and TCP biomasses at the gap of 1.00 mm. It shows the original alfalfa and original corn stover have sugar yields by 33.4% and 16. 9%, which is far higher than the sugar yield of original woody biomass (< 5%). By TCP process, the sugar yield is increased for all biomasses.

Table 5.1: Sugar yield of four biomasses with raw materials, TCP biomasses, TCDRP biomasses

Reducing sugar yield (%)	Alfalfa	Corn stover	White birch	Black spruce
Raw materials	33.4	16. 9	4.5	0.7
TCP biomasses	44.0	42.2	34.4	3.3
TCDRP biomasses	52.1	78.6	39.1	6.9

Note: TCDRP refining gap is 1.00 mm.

- Mechanical pretreatment

Table 5.1 also shows the sugar yield of the TCDRP biomasses at the gap of 1.00 mm. By an additional disk refining pretreatment, the sugar yield of corn stover and alfalfa have improved significantly by 36.4% and 8.2%, compared to the increment of by 5.1% and 3.6% for white birch and black spruce. Finally, the order of reducing sugar yield of four biomasses is as follows: corn stover > alfalfa > white birch > black spruce.

Furthermore, Figure 5.12 shows the effect of refining gap on sugar yield. The smaller gap, higher sugar yield. For example, when the gap is from 1.00 mm to 0.15 mm, the increment of sugar yield of corn stover is 16% while that of alfalfa is 22%, which is higher than that of sugar yield of woody biomasses (9% for white birch and 11% for black spruce). Moreover, at the range of gap from 0.15 mm to 0.50 mm, the reduction of fiber length increases the accessibility of enzymes (in Figure 5.8). This is primarily due to the increase specific surface area of small fibers and fines. Because the smaller fibers are easier to hydrolyze completely [94].When the gap is larger than 0.50 mm, the

fiber length doesn't change with various gaps. The effect of fiber swelling and internal structure disruption plays dominate role in improving sugar yield. According to the fiber length and shives content, corn stover and alfalfa have shorter fiber length than others at a given refining conditions. Therefore, the refining pretreatment can significantly improve the sugar yield from alfalfa and corn stover than woody biomass.

At last, corn stover reaches the highest sugar yield from 78% to 100%. That means that of the carbohydrates in pretreated biomasses are degraded into sugars. According to the lowest lignin remains in the pretreated biomasses and the shortest fiber length in corn stover, corn stover reaches the highest sugar yield than the other biomasses. In contrast, black spruce has the lowest sugar yield by the highest lignin and the longest fiber length. Therefore, the combination of lignin removal and fiber size reduction significantly affect the enzymatic hydrolysis, due to high lignin on fiber surface that blocks enzymes accessibility and specific surface of fiber by small fiber size could increase enzyme accessibility.

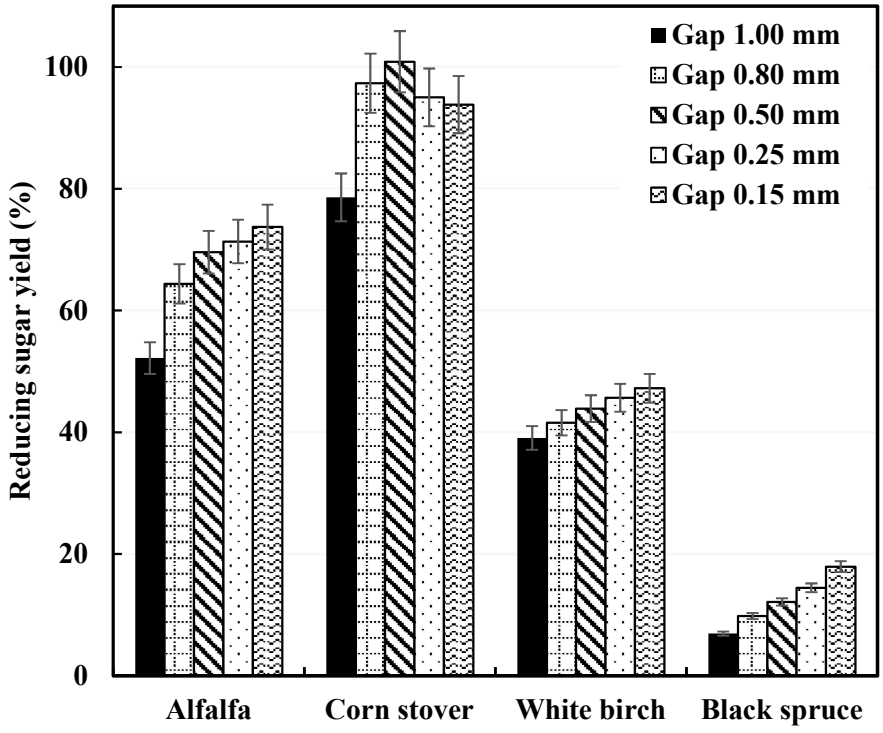

Figure 5.12: Relationships between reducing sugar yield and various lignocellulosic biomasses

5.3.4 Pretreatment energy efficiency

To analyze the pretreatment energy efficiency, Figure 5.13 shows that the effect of biomass species on pretreatment energy efficiency defined as the sugar yield on unit mechanical power consumption [11]. At first, the refining gap by 0.80 mm in the TCDRP process shows the highest sugar yield per energy consumed. Secondly, the TCDRP's energy efficiency on biomasses are presented as follows: alfalfa > corn stover > white birch > black spruce. Therefore, from an economical point of view, this pretreatment is more suitable for agricultural biomass and hardwood according to their pretreatment energy efficiency. Especially, the sugar yields of corn stover and alfalfa are 97.3% and 69.6%, where its energy consumption is 196 kWh/ton and 120 kWh/ton respectively.

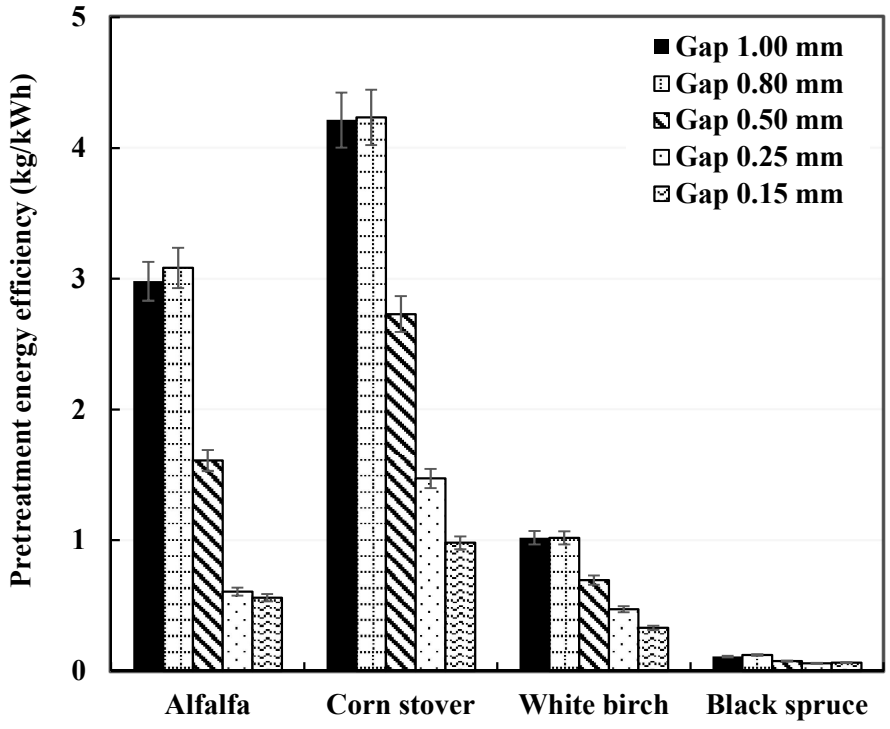

Figure 5.13: Relationships between biomass species and pretreatment energy efficiency

5.4 Conclusions

Thermochemical disk refining pretreatment was applied to four lignocellulosic biomasses: black spruce, white birch, corn stover and alfalfa.

- The extractives, hemicelluloses, lignin and cellulose during each step of the TCDRP process of four biomasses were determined. It shows that by the pretreatment process, there is a

removal of lignin and hemicelluloses by steaming pretreatment for all biomasses. From low to high, the lignin content of four biomasses are shown as follows: corn stover, alfalfa, white birch and black spruce.

- The refining pretreatment at a lowering gap consumes more energy consumption, thus reducing shives content and fiber length. At the same gap, black spruce has the highest fiber length while corn stover has the lowest fiber length.
- The sugar yield evaluation by enzymatic hydrolysis shows that the thermochemical pretreatment increases sugar yield depending on the amount of lignin removal in each biomass and final lignin content. The additional refining treatment plays more effect on corn stover and alfalfa than that on white birch and black spruce due to its size reduction. Finally, this pretreatment has more effect on agricultural biomass and hardwood (white birch). Especially, the sugar yield of the TCDRP corn stover is the highest (97.3%) compared to other biomasses, wherein its energy consumption is 196 kWh/ton.

5.5 Acknowledgement

The BiofuelNet Canada financially supported this work. We are grateful for Mr. Alain Marchand and Mr. Bryan Brousseau for their excellent assistance. We are also grateful to the sawmills and pulping mills which provided the biomasses.

CHAPTER 6 GENERAL DISCUSSION

This thesis have discussed the development and application of using the existing disk refining system as a pretreatment step to improve the yield of enzymatic hydrolysis for sugar extraction from lignocellulosic biomasses. Chapter 4 (Article 1) concentrates on the mechanical pretreatment process development. Chapter 5 considers the performance of the developed mechanical pretreatment process on different lignocellulosic biomass species. In the following discussion, the main findings, limitations, and connections between different sections of the thesis have been examined, in a general manner.

6.1 Development of disk refining pretreatment

The disadvantages of using mechanical pretreatment are high-energy cost and low sugar yield of enzymatic hydrolysis. The TMP mills have existing equipment and experienced workers, which also require alternative products without high process modification cost.

- Thermochemical pretreatment

A chemical pretreatment step, prior to the disk refining pretreatment, can increase the sugar yield in enzymatic hydrolysis with low energy consumption. However, the effect of the thermochemical pretreatment on sugar yield and energy efficiency depends on many factors such as chemical types, biomass species and pretreatment conditions. Moreover, the study of the low dilute alkaline combined disk refining pretreatment for corn stover aims to remove acetyls to achieve high sugar yields and energy efficiency while the reaction time is long at 80˚C. Besides, this technology has the drawback for the high lignin content of woody biomass since lignin is a strong inhibitor in enzymatic hydrolysis.

In article 1, our results firstly show the partial lignin removal by the diluted NaOH solution can significantly save energy consumption of disk refining and increase sugar yield of enzymatic hydrolysis of hardwood as well as shorten the reaction time at the thermal treatment (140 ˚C). The limitation of this research is lack of the results of the thermochemical pretreatment at high thermal temperatures and the steaming energy cost. This drawback can be addressed by using a steaming equipment with good conditions. In addition, the glucose yield, xylose yield and the chemical composition of waste liquid, however, not investigated and it is recommended that it can be done in future studies.

- Refining consistency and gap size

The quality of pulp and energy consumption depends on the refining conditions and other factors such as biomass species, pretreatment methods and plate patterns. The selections of disk refining conditions in SPORL pretreatment for the purpose of the feedstock of enzymatic hydrolysis have been studied for softwood biomass. The dramatically energy reduction of disk refining pretreatment can be achieved by low consistency refining (50% -3%) while slightly affects enzymatic hydrolysis. Moreover, a larger refining gap (0.38 mm-2.54 mm) reduces energy consumption with high sugar yield of softwood; and the obtained maximum pretreatment efficiency is at the gap of 0.76 mm.

In the following part of the article 1, based on the results of the thermochemical pretreatment, our results support these findings of low consistency refining and further show that 8% of the consistency is critical for enzymatic hydrolysis yield for the primary refiner for hardwood. The refining gap study also further supports the relationships among gap (the range of gap size between 0.15mm and 1.00 mm), sugar yield and energy consumption of hardwood. The modified TCDRP could achieve high sugar yield and energy consumption without new equipment adding. In addition, the sugar yield including glucose, xylose, mannose, the chemical component of waste liquid after pretreatment and steaming energy consumption should be taken into account. These results can be used to evaluate the economic value of using TMP disk refining system for biorefinery mills.

6.2 Different lignocellulosic biomass

The species of lignocelluloses have major differences of fiber structures and chemical compositions. The knowledge of the performances of the TCDRP process on the representative woody biomass, agricultural wastes and grass is limited. Especially, the alfalfa has not been widely studied for biorefinery yet. In Chapter 5, our data revealed corn stover and alfalfa could reach higher energy efficiencies of the TCDRP pretreatment than woody biomass. However, softwood has very low sugar yield by the TCDRP pretreatment technology, which is similar with the findings from other alkaline-based pretreatment methods. In addition, our results further show that the highest pretreatment efficiency of the TCDRP for corn stover and alfalfa is able to achieve at the refining gap of 0.80 mm.

CHAPTER 7 CONCLUSION, CONTRIBUTIONS, AND RECOMMENDATIONS

7.1 Conclusions

The primary objective of this thesis was achieved. A mechanical pretreatment process that is using existing disk refining system to improve the sugar yield of enzymatic hydrolysis have been developed and applied on typical lignocellulosic biomasses.

The thermochemical disk refining pretreatment (TCDRP) includes two steps: (1) the thermochemical pretreatment: the addition of NaOH by 5% w/w dry biomass and steaming pretreatment at 140 ˚C for 30 minutes; (2) the disk refining pretreatment: 8% of the refining consistency and 0.80 mm of the refining gap size. Compared to a non-modified disk refining pretreatment (typical TMP pulping conditions), the TCDRP not only improves reducing sugar yield of enzymatic hydrolysis by 35% but also saves specific energy consumption about 62%.

Using thermochemical before disk refining pretreatment can reduce power consumption and improve sugar yield of enzymatic hydrolysis. By adding 5% of NaOH (w/w dry biomass) into thermal pretreatment at 140˚C, partial lignin can be degraded, thereby increasing carbohydrates content in substrates. Net specific energy consumption of refining pretreatment can be significantly reduced by lowering refining consistency from 15% to 5% and increasing refining gap from 0.15 mm to 1.00 mm. From 15% to 5% of the refining consistency, 83% of energy consumption is saved with slightly lowering sugar yield. Refining gap size dominates fiber length, in comparison with refining consistency, which can further increase sugar yield by reducing refining gap.

The utilization of the TCDRP was experimented to various lignocellulosic biomasses: black spruce, white birch, corn stover and alfalfa. The chemical components analysis showed that there is a removal of lignin and hemicellulose by the thermochemical pretreatment for all biomasses. The reducing sugar yield of enzymatic hydrolysis revealed that the TCDRP increases sugar yield depending on its amount of lignin removal and final lignin content in each biomass. The second step pretreatment of disk refining plays more effect on corn stover and alfalfa than that on white birch and black spruce due to its size reduction.

7.2 Original contributions

The main findings of this thesis are summarized as follows:

- A novel mechanical pretreatment strategy that uses existing digester and disk refiner in TMP mills without adding new equipment was developed. It can be successfully directly applied to biochemical conversion pathways for sugar extraction.
- An alkaline-thermal treatment has been developed, which significantly enhances the quality of feedstock for disk refining pretreatment with high digestibility and low energy cost.
- An operating condition of disk refining pretreatment was developed which significantly increases sugar yield and energy saving.
- A research of the TCDRP process on different lignocellulosic biomasses have been developed, which would lead to technologically and economically possible for the disk refining pretreatment.

7.3 Recommendations

This thesis left many topics open, which are recommended for future research.

1. Investigate the impacts of thermal treating biomass at higher severity and fast pressure release, specifically, at 180 ˚C or 200 ˚C, with a retention time of 30 minutes, on the pretreatment efficiency.

This will allow a more comprehensive analysis of the impact of autohydrolysis temperature on enzymatic hydrolysis yield.

2. Investigate the impacts of using a lower amount of chemicals in the TCDRP methods on the pretreatment efficiency.

This will look at the impact of additional chemicals and their interaction during chemical treatment; it will be more economical and environmental by using a lower amount of chemicals.

3. Identify the effects of disk refining pretreatment on chemical pretreated fibers structural properties and the efficiency of enzymatic hydrolysis.

The identification will understand the mechanism of disk refining on chemical pretreated fiber. This will help us to choose the suitable pretreatment methods before the disk refining.

4. Validate the limits of the developed pretreatment process by enzymatic hydrolysis tests.

This will look at suitable enzymatic hydrolysis conditions for this feedstock from disk refining pretreatment. Further investigation is required to see if these conditions of enzymatic hydrolysis would result in higher yield, especially in the larger reactors.

5. Evaluate the technical-economical feasibility of this pretreatment process.

This would help to determine the economical possibility of scaling up the disk refining pretreatment.

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